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Oxidation -Reduction Chemistry of Methane and Nitrous Oxide in Soils and an Approach to Minimize Their Emissions From Irrigated Rice Fields.

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OXIDATION-REDUCTION CHEMISTRY OF METHANE AND NITROUS OXIDE IN SOILS AND AN APPROACH TO MINIMIZE THEIR EMISSIONS FROM IRRIGATED RICE FIELDS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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The Department of Oceanography and Coastal Sciences

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ABSTRACT

Rice fields are an important source of the greenhouse gases methane (CH_4) and nitrous oxide (N_2O). In this dissertation study, experiments were conducted at three levels - in soil suspensions, in soil cores, and under actual field conditions, to investigate the impact of soil redox potential on CH_4 and N_2O emissions to the atmosphere.

Methane and N_2O emissions occurred at distinctively different redox conditions in the homogenous soil suspensions. No significant amounts of CH_4 was produced when the soil redox potentials were above -150 mV. In the denitrification process, both N_2O production and reduction occurred in the soil redox potential range of +350 to +400 mV. When N_2O reduction was not inhibited by acetylene, N_2O tended to accumulate in the redox potential range of +120 to +250 mV. Therefore, both N_2O and CH_4 emissions were low in the general redox potential range of +120 to -170 mV where the soil was too oxidized to produce CH_4 and too reduced to produce N_2O. Nitrous oxide is a strong chemical oxidant, and the addition of N_2O to the reducing soil suspensions could result in a considerable increase of the soil redox potential.

In the heterogeneous soil cores and under field conditions, higher CH_4 concentrations were found at greater depths in the soil, while N_2O concentrations tended to form multiple peaks with depth in the soil profile. The seasonal variations of CH_4 emissions from rice fields were consistent with the development of strongly reducing conditions in the soils. Non-flooding irrigation management reduced CH_4 emissions by about 70 to 80 % in the rice growing season. A potential risk exists to increase N_2O emissions by the proposed irrigation management, but higher soil organic matter content effectively prevented the increase of N_2O emissions by facilitating N_2O reduction to N_2.

With organic manure application, the rice yields were maintained regardless of different irrigation practices during the rice growing season. Control of both irrigation and organic manure application may be a practical approach for mitigation of greenhouse gas emissions in the irrigated rice fields without adverse effects on rice yield.

CHAPTER I INTRODUCTION AND RESEARCH OBJECTIVES

GREENHOUSE EFFECT AND GREENHOUSE GASES

The greenhouse effect is essential to life on Earth. Without it, the average temperature of the surface of the Earth would not be 15 °C but -18 °C (Spiro and Stigliani, 1998). The basic theory behind the greenhouse effect is well established. Natural greenhouse gases in the atmosphere allow solar radiant energy to pass through the atmosphere to be absorbed at the Earth's surface, but trap, in the lower atmosphere, much of the infrared radiant heat emitted from the Earth's surface back toward space. These gases raise the Earth's average temperature about 33 °C higher than it would be if these gases were not present.

One of the pressing problems currently facing the Earth and its inhabitants is the concentrations of the greenhouse gases that absorb and reflect back some of the infrared radiation emitted from the Earth. These gas concentrations are definitely increasing (Figure 1.1). Since the greenhouse effect is a permanent part of the global climate system, warming from higher than natural levels of greenhouse gases should be called an "enhanced greenhouse effect." An increase in greenhouse gases may change the heat budget of the atmosphere and lead to an increase in the average surface temperature of the Earth and a change in both overall climate and climatic patterns.

According to the Intergovernmental Panel on Climate Change (IPCC, 1996), climate change is any "change in climate over time whether due to natural variability or as a result of human activity." Increasing evidence suggests the present warming, especially over the last few decades, is greater than naturally occurring climate

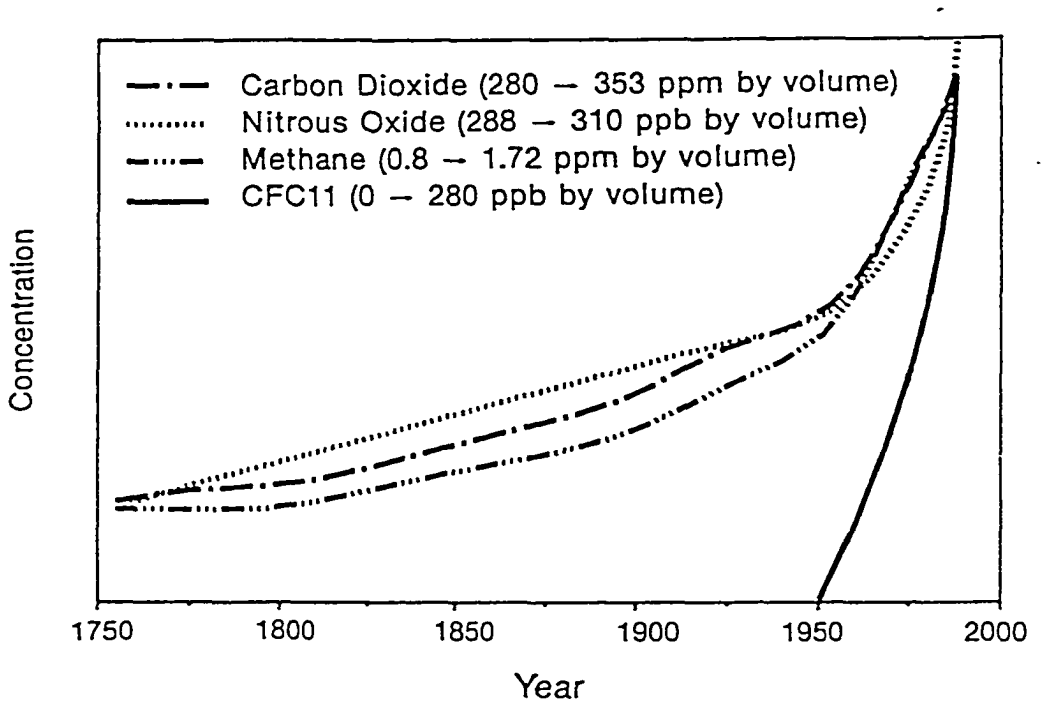


Figure 1.1 Atmospheric increases in CO_2, CH_4, N_2O and CFC-11 since 1750 (source: IPCC, 1992)

fluctuations. This phenomenon is highly significant for the recently observed 30-year temperature trend pattern. Evidence convinced the IPCC to conclude in its landmark 1995 report that recent changes in global climate trends are "unlikely to be entirely due to natural causes." There is a 95 % chance that the rise in global temperature over the past century is caused by the increase of greenhouse gases. The data are consistent, as seen in the historical records in ice cores, with the general trends expected from a greenhouse-enhanced atmosphere (Figure 1.2).

The major atmospheric gases are actually unable to absorb infrared light. They do not meet the two fundamental requirements for the absorption of electromagnetic radiation (Spiro and Stigliani, 1998):

1) When radiation is absorbed by a molecule, the molecule undergoes a quantum transition, involving the movement of either its electrons or its nuclei. The energy of the radiation must therefore match the energy of the molecular transition. In the infrared region of the spectrum, the available transitions involve movement of the nuclei in molecular vibrations. That is why argon, the third most abundant atmospheric constituent, is transparent to infrared radiation. Since argon is monatomic, it has no vibrations.
2) Because radiation is electromagnetic, its absorption requires that the transition change the electric field within the molecule, that is, the transition must alter the molecule's dipole moment. This second requirement is the reason that N_2 and O_2 are unable to absorb Earth's infrared radiation. Although their nuclei do vibrate along the bond joining them, and the energy of the vibration is in the infrared region, the vibration

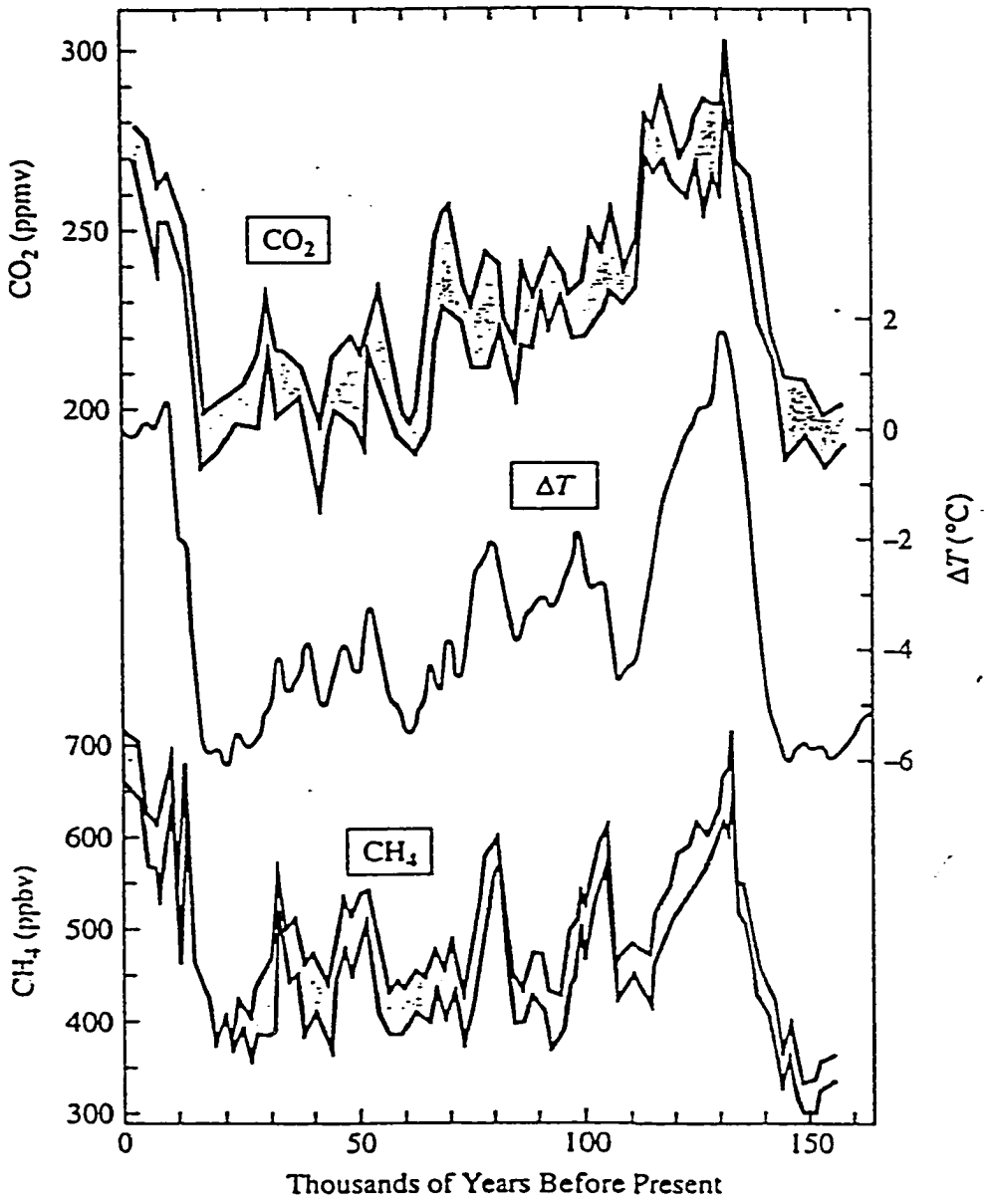


Figure 1.2 Antarctic ice core records of local atmospheric temperature, and corresponding atmospheric concentration of carbon dioxide and methane for the past 160,000 years (Source: IPCC, 1992)

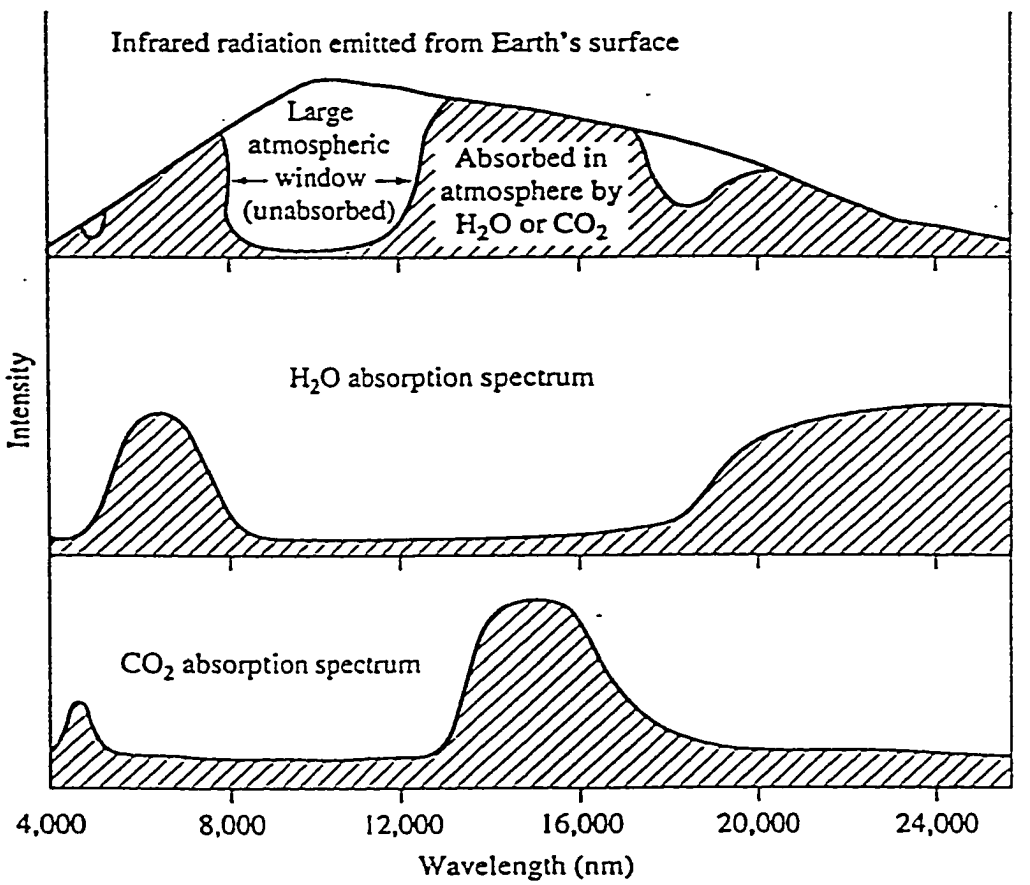


Figure 1.3 Absorption of terrestrial radiation by water and carbon dioxide (Source: Spiro and Stigliani, 1998)

does not change the dipole moment, because the molecule remains symmetrical. The vibration is infrared-inactive.

The two most important greenhouse molecules are water (H_2O) and carbon dioxide (CO_2). The combined absorption bands can be seen to block most of the terrestrial radiation (Figure 1.3). However, a relatively unobstructed region of the spectrum occurs between 8000 and 12000 nm through which most radiation can escape. This region is called the atmospheric window. This window can be filled by other greenhouse gases such as methane (CH_4), nitrous oxide (N_2O), and the chlorofluorocarbons (CFC).

The warming potential of a greenhouse gas depends on its effectiveness as an infrared absorber. Methane and N_2O are more effective because their absorption spectra are located in the atmospheric window. While the CO_2 absorption is nearly "saturated," that is, most of the radiation emitted within the absorption band for CO_2 is already absorbed. As a result, each extra CO_2 molecule contributes only a relatively small amount to the total absorption. The warming potential also depends on the residence time of the gas. The N_2O contribution per molecule is much larger than that of CH_4, because it is much longer lived.

Sources and Sinks of Methane and Nitrous Oxide

The global warming potential of CH_4 results from its atmospheric residence time of about 10 years and the fact that it is 20 to 30 times more efficient than CO_2 in trapping infrared radiation. On a 100 year time horizon, CH_4 is responsible for approximately 15-20 % of anticipated warming (IPCC, 1995).

Annually, about 540 Tg of CH_4 is emitted to the atmosphere from the biosphere (Appendix I). Of this amount, about 100 Tg arises from fossil fuels and another 100 Tg from ruminant animals and animal wastes. The remainder comes from terrestrial and aquatic systems. Biological generation of CH_4 in anaerobic environments is the principal source of CH_4 from agriculture, including enteric fermentation in ruminants (Johnson et al., 1993), flooded rice fields, and anaerobic animal waste processing. Biomass burning associated with agriculture also contributes to the global CH_4 budget. The overall magnitude of the global CH_4 emissions is reasonably well known, but estimates of CH_4 emissions from individual sources are highly uncertain. Most uncertainties arise from lack of field measurements, gaps in our knowledge on the controlling factors of CH_4 fluxes, unknown emission factors and differences in methodologies and input data to measure and estimate CH_4 fluxes (Rotmans, 1991).

The main sink for atmospheric CH_4 is oxidation with hydroxyl radicals to form CO_2 in the troposphere (Crutzen, 1981). Soil uptake and transport to the stratosphere are other important sinks of CH_4, but are small in comparison with the oxidation of CH_4 by hydroxyl radicals. Hydroxyl radicals are termed the detergents of the atmosphere, because they are responsible for the removal of almost all gases that are produced by natural processes and human activities. Any atmospheric constituents that influence the concentration of hydroxyl radicals are of considerable interest. Most aerobic soils are capable of consuming atmospheric CH_4, which provides an additional sink of 5-10 % of annual CH_4 emissions. Methane absorbed by soil is used as an energy source by some of the many microorganisms that live in the soil and either is assimilated into their body mass or evolved as CO_2 (Reeburgh et al., 1993). Consumption of atmospheric CH_4 in

aerobic soils by soil microorganisms occurs in soils globally: temperate, tropical, boreal, grasslands, and forests (Steudler et al., 1989; Mosier et al., 1991; Keller et al., 1993; Murdiyarso et al., 1996). Potential consumption by these soils is high, but the supply of CH_4 to subsurface sites of oxidation is diffusion-limited. The mechanism of CH_4 consumption is actually the same, the conversion of CH_4 to CO_2. Carbon dioxide is, of course, itself a greenhouse gas but since CH_4 has 20 to 30 times the warming effect of CO_2, its conversion to CO_2 is beneficial.

Today many of the processes controlling CH_4 fluxes are understood in detail, but required geographic information of important factors is still lacking. A major shortcoming is also the insufficient time resolution of many flux measurements to achieve representative mean seasonal fluxes. At the same time that CH_4 is being produced, it is being destroyed. About 85 % of this destruction takes place in the atmosphere by hydroxyl radicals, but a significant part of it occurs through the activities of microbes in aerobic soil. A decrease in the consumption of CH_4, due to changes in the way that we manage land, may also have contributed to the increase in atmospheric CH_4 concentration. Methane is increasing in the atmosphere at the rate of about 1.2 % $year^{-1}$. The increase of CH_4 over the past 200 years is probably due to the increase of emissions (70 %) while about 30 % may be caused by the depletion of hydroxyl (OH) radicals (Khalil and Rasmussen, 1990).

Although N_2O occurs in the atmosphere in minute quantities compared to CO_2 and water vapor, its contribution to the greenhouse effect is considerable. This effect is caused by its long residence time in combination with the relatively large energy absorption capacity per molecule. Per unit mass of N_2O, the global warming potential is

about 310 times greater than that of CO_2. Nitrous oxide is increasing in the atmosphere at the rate of about 0.25 % $year^{-1}$. It has an atmospheric residence time of about 120 years, and over a projected 100 year time horizon, is anticipated to be responsible for about 5 % of expected warming (IPCC, 1995). Total annual emissions of N_2O from the biosphere to the atmosphere were approximately 15 Tg (Appendix II). The major sources in the N_2O budgets are soils under natural vegetation, followed by oceans. Production of N_2O in soils and emission to the atmosphere account for about 70 % of both anthropogenic N_2O and natural N_2O sources. Despite the uncertainty in the global N_2O budget, the most recent IPCC (1995) assessment indicates that agricultural activities are the most important anthropogenic source of N_2O.

Most of the N_2O in the Earth's atmosphere stems from microbiological processes. In soils and aquatic systems, the major sources of N_2O are generally accepted to be nitrification and denitrification. Simply defined, nitrification is the aerobic microbial oxidation of ammonium to nitrate, and denitrification is the anaerobic microbial reduction of nitrate to dinitrogen gas. Nitrous oxide is an intermediate product in the reaction sequences of both processes that leaks from microbial cells into the soil atmosphere (Firestone and Davidson, 1989). In well-aerated soils, N_2O emissions as a result of nitrification of ammonium can be substantial (Linn and Doran, 1984). In wet soils, where aeration is restricted, denitrification is generally the source of N_2O (Smith, 1990). In such oxygen (O_2) limited conditions, both the rate of denitrification and the N_2O:N_2 ratio must be known to evaluate N_2O emissions through denitrification (Mosier, 1998). Soil structure, water content, microbial populations, and available C are important factors

determining the proportions of the two gases, and affect the balance between diffusive escape of N_2O and its further reduction to N_2.

The only known significant sink for N_2O in the atmosphere is movement into the stratosphere where it is photolyzed to NO. Under reducing conditions with no other available source of N, N_2O may be consumed in soils. Uptake of N_2O by the ocean surface has also been observed. At present knowledge of conditions at which soils and aquatic systems act as sinks for N_2O, and the parameter affecting the influx when they do so, is too limited to evaluate their importance at the global scale.

METHANE AND NITROUS OXIDE ON OZONE DESTRUCTION

Concern about pollution of the stratosphere centers on possible threats to the ozone (O_3) layer. Ozone serves two essential functions: it protects living organisms on Earth from the harmful effects of the sun's ultraviolet (UV) radiation, and it provides the heat source for layering the atmosphere into a stratosphere and a troposphere. Ozone changes affect the UV flux most sensitively at the shortest wavelengths where the damage to biological molecules is the greatest. A 1 % decrease in the ozone layer gives a 1 % increase in ultraviolet transmission at 310 nm, a 3 % increase at 300 nm, and 10 % at 290 nm (Spiro and Stigliani, 1998).

Nitrous oxide is a long-lived gas because it is inert in the troposphere. However, above 30 km in the stratosphere, most of the N_2O is photolyzed by UV photons to produce dinitrogen and excited O_2 atoms. A small percentage, 10 % or less, of the N_2O molecules react with excited O_2 atoms to produce nitric oxide (NO). This is the main source of NO in the stratosphere. Nitric oxide is one of the catalysts involved in a chain of reactions that deplete O_3. The yield of NO through N_2O oxidation provides the major

input of NOx to the stratosphere, thus in part regulating stratospheric ozone and influencing the NOx balance in the upper troposphere (Crutzen, 1970). Although NO is produced abundantly in the lower atmosphere by combustion and lightning, almost all of it is oxidized to NO_2 and converted to nitric acid in the troposphere, after which it is rained out before reaching the stratosphere. On the other hand, N_2O, although much less abundant, is also much less reactive, and does eventually reach the stratosphere.

Methane also influences the chemistry of the stratosphere. Its oxidation is an important source of stratospheric water vapor, and so directly of hydroxyl radicals. Stratospheric CH_4 can react with Cl radicals, forming HCl that slows the rate at which Cl and ClO destroy stratospheric ozone (Crutzen, 1981).

A broad perception exists that the science of global warming is much less certain than the science of stratospheric ozone depletion. However, the level of uncertainty surrounding the ozone problem is not much different than it is for global warming.

Methane and Nitrous Oxide Emissions from Rice Fields

Isotopic measurements of atmospheric CH_4 show that 70-80 % is of biogenic origin (Wahlen et al., 1989). About 40 % of the roughly 500 Tg of CH_4 produced annually comes from soils. Physical environment exerts a major control on CH_4 emissions, thus the prevalence of waterlogged, anoxic conditions favors production of CH_4. Natural wetlands occupy approximately 500-600 Mha and emit about 100 Tg of CH_4 annually (Matthews, 1993). Flooded rice fields occupy about 148 Mha, produce about 475 Mt $year^{-1}$ of rice, and emit about 50 Tg of CH_4 annually (Cole et al., 1996). In total, natural wetlands and wetland rice fields account for about one third of the total global estimated annual CH_4 source (IPCC, 1992).

Flooded rice fields are significant sources of atmospheric CH_4. The emission of CH_4 is the net result of opposing bacterial processes, production in anaerobic micro-environments, and consumption and oxidation in aerobic micro-environments (Bouwman, 1990). The relative source intensity of CH_4 (annual average CH_4 emission rate per unit area) in rice ecosystems follows the general order: irrigated rice > favorable rainfed rice > flood prone rainfed rice > deepwater rice > drought prone rainfed rice > tidal wetland rice. Upland rice is not a source of CH_4 since it is grown like wheat in aerated soils that never become flooded for a significant period of time. Irrigated rice has the highest CH_4 source intensity because of the assured water supply and the area planted. Differences in residue recycling, organic amendments, scheduled short aeration periods, soils, fertilization, and rice cultivars are major causes for variations of CH_4 fluxes in irrigated rice. Highest CH_4 fluxes are observed in fields receiving organic amendments. Lowest CH_4 fluxes are recorded in fields with low residue recycling, multiple aeration periods, poor soils and low fertilization with resulting poor rice growth and low yields (Harriss, 1993). Understanding and modeling of processes have progressed well and large-scale information on rice growing areas, growing seasons, temperature regimes, and soil types is available. Essential geographic information on water regimes, organic recycling and amendments, controlling soil properties, rice cultivars, and cultural practices is still insufficient or not available at present.

Although high uncertainties exist about the sources and sinks of CH_4 and N_2O, irrigated rice fields have been identified to be an important source of CH_4 in the flooded season, and of N_2O in the unflooded season, because both aerobic and anaerobic environments exist in these soil-plant-water systems. Many physical, chemical and

biological factors influence CH_4 and N_2O production and emission. Soil water content determines soil aerobic and anaerobic condition, which is indicated by soil redox potential (Eh). Favorable redox potential is essential for both CH_4 and N_2O production and emission from rice fields. An oxidized flooded soil surface layer maintained by the O_2 in the flooding water, and an oxidized rice plant rhizosphere maintained by O_2 diffusing through the plant have been identified to be important in regulating CH_4 flux in rice fields. A significant amount of CH_4 produced in soils will be oxidized in these two zones before it escapes from the soils into the atmosphere. Nitrification will also readily occur in these two aerobic zones, by which ammonium will be converted to nitrate. This nitrogen transformation process will provide N substrate for denitrification to function, part of which can stop at the intermediate N_2O step, and also will lead to enhanced leaching loss of N in aerobic soils because of the higher mobility of nitrate than that of ammonium. Organic matter can increase soil O_2 consumption activity, which might decrease the oxidized rhizosphere volume and make the flooded aerobic surface layer thinner. Proper irrigation practices, organic matter management, and possibly the type of nitrogen fertilizer used might be practical approaches to minimizing N_2O and CH_4 emissions.

RESEARCH OBJECTIVES

The atmosphere is a repository for emissions from many different natural and human activities. The air can be cleansed by natural mechanisms, but these can be overwhelmed by the amounts of pollutants being produced. The global enhanced warming is just beginning to be addressed, and it is much more difficult to solve than regional problems. Much of the focus is on CO_2, and little attention has been given to the

other trace gases such as CH_4 and N_2O. It is a great challenge to keep a sustainable development in agriculture, like in rice production. On one hand, increasing population needs intensive agriculture to make enough food and other products, which needs increasing fertilizer applications. On the other hand, intensive agriculture and fertilizer applications are a major anthropogenic source of CH_4 and N_2O.

There are two main interrelated research areas in this field: (1) to better understand the processes and factors that affect CH_4 and N_2O production and emission, and, (2) to find some possible management practice in fields using current knowledge to minimize greenhouse gas emissions, increase fertilizer efficiency, but not lower crop yields. This dissertation study examined both the production of CH_4 and N_2O and their consumption (reduction of N_2O and oxidation of CH_4). The goal of this study is to gain further insights on how soil redox potentials control CH_4 and N_2O production and emission, and try to identify the soil redox potential range where both CH_4 and N_2O emissions are low. This information should result in recommendations for modified agricultural management practices to minimize CH_4 and N_2O release, such as irrigation management to maintain soil redox potential in a desirable range. It is expected to find an optimum combination of fertilization, organic matter application, and irrigation management that can minimize both CH_4 and N_2O emissions from irrigated rice fields, but will not decrease crop yields.

To accomplish these research objectives, experiments were conducted at three levels: a laboratory soil suspension study, a soil core incubation study, and a field study. Methane production under different soil redox conditions have been well documented. Chapter II is a literature review to discuss on significant CH_4 production under strictly

anaerobic conditions and early production of trace amount of CH_4 at higher redox conditions. There is a considerable lack of information on N_2O production and reduction in relation to soil redox status. Chapter III presents the findings of a laboratory study on this topic. In Chapter IV, both CH_4 and N_2O are covered in the same experiment in order to identifiy a soil redox potential range where both gas emissions are comparatively low. Nitrous oxide is generally recognized as an reductant following nitrate or nitrite reduction. Both experimental and literature evidence is provided in Chapter V to indicate that N_2O is a strong oxidant that has profound implications on soil oxidation-reduction chemistry. A soil core incubation study in Chapter VI provides a link between soil suspension experiments and field measurements. The field study in Chapter VII was conducted in China, and covers both CH_4 and N_2O emissions from rice fields with different treatments for irrigation and organic matter application. This study is the first trial known designed to control soil redox potentials in fields in order to minimize CH_4 and N_2O emissions. Chapter (VIII) presents anticipated future research opportunities, especially regarding N_2O.

CHAPTER II METHANOGENSIS AND ITS RELATION TO SOIL OXIDATION-REDUCTION CONDITIONS

General Methanogensis

Biological methanogensis, carried out by a mixed culture of bacteria to convert complex forms of organic matter into CH_4 and CO_2, is an exceedingly common and widespread process in nature. The process requires a rather specific set of environmental conditions, including the presence of suitable energy-yielding substrate, the usual nutrient elements, a pH near neutrality, a low redox potential, and a sufficiently low concentration of inhibitory compounds. Biological methanogenesis is capable of converting almost any organic substrate nearly quantitatively into a mixture of CH_4 and CO_2 (Updegraff, 1980). Only a few reactions leading directly to the production of CH_4, and two distinct metabolic pathways for biological formation of CH_4 have been identified (Takai, 1970; Zeikus, 1977):

1) Carbon dioxide reduction, utilizing H_2 gas, fatty acids or alcohol as a H_2 donor. This reaction is carried out by all cultures of methanogenic bacteria isolated to date. The direct precursor of CH_4 has been identified by McBride and Wolfe (1971) as methyl coenzyme M. It is the reduction of this compound that leads to the production of CH_4.

   CO_2 (aq.) + $4H_2 = CH_4 + 2H_2O$ $\Delta Gr^0 = -139$ kJ (at pH 7)

2) Transmethylation of acetic acid or methyl alcohol, not involving CO_2 as an intermediate. This methanogenic reaction is the splitting of acetate. Methane comes from the methyl group of acetate and the fourth H from water.

   $CH_3COO^- + H_2O = CH_4 + HCO_3^-$ $\Delta Gr^0 = -28.2$ kJ (at pH 7)

Many of the acetotrophic methanogens so far isolated are able to use H_2 instead of acetate, and with some of them, e.g. strains of *Methanosarcina*, acetate degradation is even inhibited by H_2. On the other hand, methanogens that have been isolated with H_2/CO_2 are generally unable to use acetate for methanogenesis (Conrad et al., 1987). Since CH_4 is formed only from CO_2 and acetate (Rajagopal et al., 1988; Ferguson and Mah, 1983), the activities of other microorganisms must be invoked to supply these metabolites. Methane is also produced from formate and methanol by some species, but this process does not contribute considerable amounts of CH_4 under natural conditions.

INHIBITION OF METHANOGENSIS

Methanogen Population

The CH_4-producing bacteria are strict anaerobes that require a very low redox potential before they can initiate growth. Under natural conditions the other facultative and obligate anaerobes present perform the function of consuming all available O_2 and then producing a strongly reducing environment in order to permit growth of the methanogens. Although the methanogens are incapable of growth in the presence of dissolved O_2, Zehnder (1978) has shown that at least one pure strain, *Methanobacterium* strain A_2, is not killed even by high O_2 concentrations. Thus methanogenesis can begin again soon after it is inhibited by O_2 since facultative anaerobes in the environment are capable of rapidly depleting the dissolved O_2 if the supply rate is not excessive.

Oxygen release from rice roots controls methanogenesis in the rooted upper soil layer either directly or by the oxidation of ferrous iron. The presence of ferric iron, resulting from the input of O_2 via the roots, results in a shift of electron flow from methanogenesis to ferric iron reduction. Frenzel (1999) found no difference or change

present in the numbers of culturable methanogens between the rice rooted upper soil layer and unrooted lower layer, either at the beginning or at the end of the experiment. Although the methanogenic bacteria are strict anaerobes and do not form spores or other resting stages, they are obviously able to survive the periods when the rice soil is dry and oxic between the flooding periods. Exposure to O_2 had an additionally detrimental effect on the viability of methanogenic bacteria and on the potential of the treated cells to produce CH_4 (Fetzer et al., 1993). Kiener et al. (1988) discussed the biochemical mechanism on the reversible conversion between the coenzymes related to CH_4 production upon O_2 exposure.

The methanogenic population in rice fields stays constant during dry fallow periods. Even in forest and arable soils, a very small methanogenic population exists that can become active under anoxic conditions and produce CH_4 (Mayer and Conrad, 1990). Peters and Conrad (1995) found that strictly anaerobic bacteria, such as methanogenic, sulfate-reducing, and homoacetogenic bacteria, could be enriched from all tested oxic soils in their study. The detection of methanogenic bacteria was especially surprising because in contrast to sulfate-forming or homoacetogenic bacteria, there are no spore-forming or other known resistant species. The fact that methanogenic bacteria can survive incubation in an O_2-containing atmosphere was also supported by other studies (Fetzer et al., 1993; Kiener and Leisinger, 1983).

Methanogen Activities

Early studies showed that substances found to be inhibitory for methanogensis include organic acids, ammonia, certain heavy metals, sulfide, sulfate and nitrate (Updegraff, 1980). Among them, inhibition by sulfate has been most extensively

investigated. Methane production does not reach an appreciable concentration until most of the sulfate is removed from soil and water systems by sulfate-reducing bacteria. Winfrey and Zeikus (1977) found that methanogenesis was inhibited by the addition of as little as 19 μg sulfate ml^{-1}. The inhibition was reversed by the addition of either H_2 or acetate. This occurrence indicates that competition for available H_2 and acetate between sulfate reducing bacteria and the methanogens is responsible for the inhibitory effect of sulfate. A similar result was found where methanogensis was found to be inhibited by an addition of 0.2 mM sulfate, and the same inhibitory mechanism was proposed (Conrad et al., 1989). The sulfate reducer population has a half-saturation constant for H_2 uptake of 141 Pa versus 597 Pa for the methanogen population. When sulfate is not limiting, the lower half-saturation constant of sulfate reducers enables them to inhibit CH_4 production by lowering the H_2 partial pressure below levels that methanogens can effectively utilize. A significant part of the anaerobic turnover of H_2 in anoxic environment is due to other microbial processes other than methanogenesis or sulfate reduction, such as homoacetogenesis that plays a significant role in the anaerobic turnover of dissolved H_2 at least in some aquatic methanogenic ecosystems. The in situ partial pressures of H_2 in rice soil are usually in a range of 1-4 pa. These H_2 concentrations are just within the range of the H_2 thresholds measured in pure cultures of H_2-utilizing methanogens (2-10 Pa) but are significantly lower than those of homoacetogens (43-95 Pa). Hence, homoacetogens should not be able to utilize the in situ H_2 (Lovley et al., 1982).

Ferric iron (Fe III)-reducing organisms can inhibit sulfate reduction and CH_4 production by outcompeting sulfate reducers and methanogens for electron donors, H_2

and acetate (Lovley and Phillips, 1987). Nitrate was shown to be a more effective inhibitor than MnO_2 for CH_4 production. In contrast, air addition did not significantly affect CH_4 formation. Primary effect of nitrate addition on reducing CH_4 production was through the resultant increase in soil redox potential (more detail discussion is included in Chapter V). Using methyl fluoride, a CH_4 oxidation inhibitor it was found that added nitrate was not used in CH_4 oxidation by methanotrophic bacteria (Jugsujinda et al., 1995 and 1998).

The study of combined denitrification and methanogenesis has been attempted in some studies. It has been observed that nitrate inhibits methanogenesis and consequently under completely mixed conditions the two processes do not proceed simultaneously. Methanogenesis was found to commence after the complete reduction of nitrate (or nitrogen oxides). Thus, the inhibition of methanogenesis by nitrate is reversible (Akunna et al., 1998). These authors ruled out the competition for organic carbon between the denitrifiers and the methanogens as the possible cause of the inhibition because their studies were carried out with organic carbon concentrations well in excess of denitrification requirements. The presence of nitrate has a reversible inhibition effect on CH_4 producing bacteria. The fact that acetic acid was present at the beginning of the experiment suggested that this inhibition affected both the acetoclastic methanogens (that convert acetic acid to CH_4) and the hydrogenophilic methanogens (that convert CO_2 and H_2 to CH_4). The presence of surplus organic carbon reduced the likelihood of competition between the nitrate-reducing bacteria and the CH_4-producing bacteria

The inhibition of methanogenesis by sulfate, Mn (IV) and Fe (III) reducing bacteria was due to the competition for their common substrate H_2 (Abram and Nedwell,

1978; Achtnich et al., 1995; Lovley et al., 1982). The same mechanism can not be excluded for the inhibition of CH_4 production by N-compounds. Nitrate and its denitrification products have been shown recently to inhibit methanogenesis by pure methanogenic strains. Addition of each of the N-compounds (nitrate, nitrite, NO and N_2O) caused a complete but largely reversible inhibition of methanogenesis. The different N-compounds (nitrate, nitrite, NO and N_2O) inhibited H_2-dependent methanogenesis to different extents. Both reversible and irreversible inhibitions on CH_4 production were found, depending on the type of methanogenic bacterium and the applied concentration of the N-compound (Kluber and Conrad, 1998a and b). Belay et al. (1990) found that nitrate could serve as a nitrogen source for growth in several methanogenic bacteria but could cause inhibition in others. Fischer and Thauer (1990) showed that N_2O inhibits CH_4 formation from acetate in *Methanosarcina barkeri*. Balderston and Payne (1976) showed that *Mb. Thermoautotrophicum* was less susceptible to inhibition by nitrate, nitrite, NO and N_2O than *Mb. Formicicum*.

Critical Redox Potentials for Initiating Methanogensis

Significant Methane Production

Soil redox status has a controlling influence on CH_4 formation. It has been determined that methanogenic bacteria in soil can function only below a certain level of redox potential because they are obligate anaerobes and require highly reduced conditions for growth (Cicerone and Oremland, 1998). Soil oxidation-reduction reactions consist of different soil oxidants (O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-} and CO_2) used as electron acceptors for organic matter degradation. The reduction of various oxidants in homogeneous soil suspensions occurs sequentially at corresponding soil redox potential values. The rapid

initial decrease of redox potential in some anaerobic incubations is apparently due to the release of reducing substances accompanying O_2 depletion. The minimum redox potential can be as low as -420 mV, and can be accompanied by the evolution of H_2 (Ponamperuma, 1972). Hydrogen is a key intermediate during the degradation of organic matter in anaerobic biotic environments, and is consumed by methanogenic, sulfate-reducing, and homoacetogenic bacteria. Since H_2 concentrations are usually extremely low in anaerobic environments, methanogenic bacteria are commonly outcompeted for H_2 by others that utilize trace amounts of H_2 more effectively (Cord-Ruwisch, 1988).

Because of the inhibition of methanogensis (both methanogen population and activities) by other oxidants, significant CH_4 production can only occur when such inhibition is released indicated by a critical level of low redox potential. The initial redox potential level of methanogenesis was reported to be as low as -300 mV by Cicerone and Oremland (1988), but higher redox potential (about -120 mV) for methanogenesis initiation was found by Jakobson et al., (1981). Based on a limited number of observations, soil redox potential of -150 mV was considered to be the critical value for initiation of CH_4 production in a Louisiana rice soil (Masscheleyn et al., 1993). In fact, the critical soil redox potential for initiation of CH_4 production is soil specific. It has been observed in a Louisiana (U.S.A.) rice soil that the critical redox potential for CH_4 production was approximately from -150 to -160 mV (Wang et al., 1993), -150 mV for a Chinese rice soil, and around -200 mV for two Belgian upland soils (Yu et al., in press). It is important to point out that the above critical redox potential was based on the vigorous CH_4 production that had been found exponentially related to soil redox potential.

Early Initiation of Methanogensis

Methane production was observed during the initial phase of anoxia in rice soil slurries despite a high redox potential and the presence of oxidants. The lack of inhibition by methyl fluoride of the early CH_4 production suggested that most of the CH_4 production at the beginning of the incubation was caused by hydrogenotrophic methanogens (thermodynamic favorable). In later incubations, a shift from hydrogenotrophic to acetoclastic methanogenesis as the dominant source of CH_4 occurred (Roy et al., 1997).

Studies on 16 different rice fields showed a general three-phase pattern related to CH_4 production (Yao and Conrad, 1999). In the first phase, exergonic methanogenesis with H_2 and acetate occurred at positive redox potential range (360-510 mV). In the second phase, due to the increase of Gibbs free energy for H_2/CO_2-dependent methanogenesis, sulfate reduction or reduction of Fe (III) became dominant. The third phase, CH_4 was vigorously produced by acetate-dependent methanogenesis and eventually accumulated with a constant rate until the end of incubation (Jetten et al., 1990). In a few soils, the initial CH_4 production (first phase by H_2/CO_2-utilizing methanogens) was not inhibited either by the high redox potential or by the presence of inorganic oxidants such as Fe (III) and sulfate so that these soils released CH_4 right from the beginning of submergence until the end. Accumulation of CH_4 started at different times depending on the soil tested. In many cases, the beginning of CH_4 accumulation approximately coincided with the end of sulfate and iron reduction. But in logarithm scale, all the tested rice fields showed such an early initial CH_4 production.

However, the initial CH_4 production was not observed in upland soils (forest, agricultural, savanna and desert soil) that had no history of CH_4 production. This probably is because the initial population size of the methanogenic bacteria is very small in the upland soils, but relatively large in the wetland rice soils. The production of CH_4 was found to be paralleled with the increase of methanogenic bacterial population. This process started at measured redox potentials of 0 to +70 mV (Peters and Conrad, 1996). The presence of methanogens and the evolution of H_2 at the beginning of soil submergence make early initiation of methanogensis thermodynamically possible, but it is not important in term of the quantity of CH_4 produced.

Soil redox potential reflects the reducing intensity of the soil and the relative oxidant/reductant contents, and can be a good and practical indicator to predicate significant CH_4 production (Stumm, 1967). Most typical soil oxidants have been found to inhibit methanogensis to some extend. Some redox sensitive substances may change the soil redox potential but not affect CH_4 production. Fetzer and Conrad (1993) found that the rate of CH_4 production were not significantly affected when the redox potential of an anoxic medium was adjusted to values between -420 mV and +100 mV by addition of titanium (III) citrate, sodium dithionite, flavin adenine dinucleotide, or sodium ascorbate. *M. Barkeri* was able to reduce 0.5 mM ferricyanide solution at +430 mV within 30 min to a value of about +50 mV, and then to start CH_4 production. The bacteria were able to decrease the positive redox potential by themselves and started methanogenesis as soon as the redox potential had decreased beyond a critical value of +50 mV. Then, the CH_4 production activity operated immediately at full rate. The ability of *M. Barkeri*, and probably also of other methanogens, to generate its own redox environment may be one

explanation of the relatively good survival of methanogenic bacteria in dry and oxic soil (Mayer and Conrad, 1990; Fetzer et al., 1993).

Conclusion

High levels of redox potentials and typical oxidants in soils and sediments demonstrate an effective inhibition on methanogensis, large amounts of CH_4 production can occur only when such inhibition is released indicated by a critical low point of redox potential level. When CH_4 concentrations were plotted on a linear scale versus soil redox potential , CH_4 production occurred mostly after the complete reduction of sulfate by sulfate-reducing bacteria. However, when the same CH_4 concentrations were plotted on a logarithmic scale, a small CH_4 production immediately occurred after the onset of anoxic conditions, even while the redox potentials were positive and in the presence of oxidants. The early initiation of methanogenesis has simply been overlooked, as it becomes evident only when CH_4 is analyzed sensitively and is plotted on a logarithmic scale. Early initiation of CH_4 production is thermodynamically possible and might be important in theory, but it could not account for the major part of CH_4 production.

CHAPTER III CRITICAL REDOX POTENTIALS FOR NITROUS OXIDE PRODUCTION AND REDUCTION

INTRODUCTION

Wetlands are characterized by an aerobic surface soil layer maintained by O_2 in the water column and an underlying anaerobic layer into which the O_2 does not penetrate. The close interface between these two layers facilitates transport of elements of different forms, and functions as a link of various chemical biological oxidation-reduction (redox) reactions. The plant rhizosphere forms the other aerobic-anaerobic interface in the soil profile. Various soil oxidants, such as oxygen and nitrate, can accept electrons from soil organic matter degradation to complete the oxidation and reduction reactions. In flooded soils, these aerobic-anaerobic interfaces influence and often controll the oxidation-reduction reactions, including nitrification-denitrification, redox cycling of iron and manganese compounds, sulfate reduction and sulfide oxidation, and CH_4 formation and oxidation (Ponnamporuma, 1972; Patrick and DeLaune, 1977). The reduction of major soil oxidants in homogeneous soil suspensions occurs sequentially with decreasing redox potential, and such reaction sequence is consistent quite well with the order of their oxidation-reduction potentials as listed in Table 3.1.

Denitrification is the major reduction process of N oxides in soils and plays an essential role in the global nitrogen cycle. Nitrous oxide is an intermediate product of denitrification, and one of the most important trace gases contributed to global warming (Dickinson and Cicerone, 1986) and destruction of stratospheric ozone (Crutzen, 1981;

Weiss, 1981). The denitrification process is generally illustrated as follows (Tiedje, 1982):

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O \longrightarrow N_2$$

In an anaerobic incubation, acetylene in 10 % of the headspace volume can effectively inhibit N_2O reduction to N_2, making N_2O an end product of denitrification for convenient detection by gas chromatography.

Soil oxidation-reduction conditions play a fundamental role in the denitrification reaction. A redox potential of approximately +200 mV was shown to be critical in order for denitrification to occur in a Louisiana soil suspension (Kralova et al., 1992). Smith et al. (1983) found in their study with a silt loam soil that a redox potential of +250 mV was the critical value for N_2O production. It is generally believed that the redox potential for the reduction of N_2O is lower than that for the reduction of nitrate in the denitrification. Nitrous oxide is commonly found to accumulate in the early phase of the denitrification process, and an accumulation of N_2 following the decrease of N_2O concentration after the maximum accumulation. However, it is important to note that this is just a substrate and product relationship of the reactions in the denitrification, instead of a redox-controlled reaction sequence. At the beginning of the incubation, N_2O is not present in the system. It depends on the reduction of nitrate, with N_2O as an intermediate product, to provide substrate for the reaction of N_2O reduction to N_2. Some studies could not find a distinct difference in the corresponding soil redox potential of these two reduction reactions. Letey et al. (1980) found a redox potential in the range of +200 to +300 mV to be critical for N_2O production and reduction in a sandy soil. Which reduction reaction will precede earlier following the decrease of soil redox potentials when both nitrate and N_2O are

Table 3.1 Oxidation-reduction (redox) potentials of major soil oxidants at different pH

Reduction process	Eh (mV) at 25 °C		
	pH = 6	pH = 7	pH = 8
$O_2 + 4\ H^+ + 4\ e^- = 2\ H_2O$	+874	+815	+755
$2NO_3^- + 12\ H^+ + 10\ e^- = N_2 + 6\ H_2O$	+815	+744	+674
$MnO_2 + 4\ H^+ + 2\ e^- = Mn^{2+} + 2\ H_2O$	+520	+401	+283
$Fe(OH)_3 + 3\ H^+ + e^- = Fe^{2+} + 3\ H_2O$	-6	-183	-361
$SO_4^{2-} + 8\ H^+ + 8\ e^- = S^{2-} + 4\ H_2O$	-144	-218	-292
$CO_2 + 8\ H^+ + 8\ e^- = CH_4 + 2\ H_2O$	-185	-244	-304

Eh is calculated according to Nernst equation

$$Eh = E^\circ - 2.303RT/nF \log [\text{Reductant}]/[\text{Oxidant}]$$

E°, standard redox potentials were cited from Handbook of Chemistry and Physics (Lide, 1991).

present is still an unanswered question. The standard redox potential of N_2O/N_2 pair is 1770 mV, even higher than that of O_2/H_2O (1229 mV). In this experiment we are trying to verify the hypothesis that N_2O reduction can occur at higher redox conditions than nitrate reduction.

MATERIALS AND METHODS

Two rice soils and two upland soils, as documented in Appendix III, were used in this experiment. Each soil was incubated in a single microcosm (Appendix IV) with no stirring or exposure to O_2 for one month. This allowed time for denitrifying enzymes to develop (Rudaz et al., 1991; Dendooven and Anderson, 1995). The same set of microcosms were run three times but treated differently each time as shown in Table 3.2. The microcosms were continuously incubated with no further O_2 supply. The redox potentials of the soil suspensions were monitored by two platinum (Pt) electrodes. During the experiment, the microcosms were purged with pure N_2 the day before each sampling date that was determined when soil redox potential changed substantially during the incubation. For treatment A and B, the accumulated gas in the headspace after purging and continued incubation for 1 day was withdrawn by using a syringe, and transferred into an evacuated vial (10 ml Vacutainer, Becton Dickinson, New Jersey, U.S.A.). Samples were taken 3 or 4 times within the day when external N_2O was added in treatment C so that the N_2O reduction rates could be calculated by linear regression of these measurements. The incubation of each soil suspension was run in single with gas sampling in duplicate.

Nitrous oxide was analyzed by a Tremetrics 9001 gas chromatography with an electron capture detector (ECD). The emission rates of N_2O were calculated by the

Table 3.2 Different treatments in the experiment

Treatment	Dextrose (1 %)	Nitrate (50 mg N kg^{-1})	Acetylene (10 %)	Nitrous Oxide (10 mg N kg^{-1})
A	YES	YES	NO	NO
B	YES	YES	YES	NO
C	YES	NO	NO	YES

Notes for the experiment treatment:

1) Same set of soil and microcosms were used in this study. The experiments with different treatments were conducted by flushing the microcosm with air to oxidize the soil back to the original oxidizing condition after completing one treatment, because all of the additions are removable from the system;

2) For all treatments organic matter, as an energy source for the microorganisms in soils, was provided by adding 4 g dextrose to each microcosm;

3) For treatment A and B, potassium nitrate was added in an amount of 50 mg N kg^{-1} soil by weight to provide substrate for denitrification;

4) For treatment B, 60 ml pure C_2H_2 was injected using a syringe into the headspace of the microcosms, and this addition was repeated whenever the microcosms were purged with N_2;

5) For treatment C, 2 ml of 98 % N_2O (with 2 % N_2) was injected through the rubber stopper, and this addition was also repeated after purging of microcosm with N_2.

amount of N_2O accumulation divided by the accumulation time and the amount of soil used in the microcosm. Redox potential values and N_2O emission and reduction rates were reported in means of duplicate measurements.

RESULTS AND DISCUSSION

The intensity of soil reduction as measured by redox potential (Eh) has been shown to be an important factor affecting both nitrification and denitrification rates. Emission of N_2O can occur from the nitrification reaction in aerobic soils where soil redox potentials are generally above +400 mV (Bremner and Blackmer, 1978; Bedard and Knowles, 1991). When soils are inundated with water, demand of O_2 by micro-organisms and plant root respiration rapidly depletes the remaining O_2. Then various chemical and biological transformations take place resulting in a decrease in redox potential. Moderately reduced soils are characterized by a redox potential range of +100 to +400 mV (Gambrell and Patrick, 1978). In most reduced (anaerobic) soils, the redox potential ranges from around -300 to +100 mV.

To make the results simple to interpret, the measurements in this experiment were carried out in a redox potential range of +400 to 0 mV where denitrification is the dominant biological process producing gaseous N products.

Estimation of the Critical Redox Potential for Nitrous Oxide Production

Nitrous oxide accumulation in the absence of C_2H_2 is the combined result of N_2O production and reduction. The N_2O production rate is greater than that of N_2O reduction until N_2O accumulation reaches the maximum when the rates of N_2O production and reduction are equal. Many factors, such as nitrate content, pH and redox status, can influence both of these activities. In this study, the highest accumulation of N_2O was

found at different redox conditions among the four tested soils (Figure 3.1). The importance of soil redox potential for N_2O production during denitrification has been reported by Letey et al. (1980) and by Smith et al. (1983). In both studies it was observed that the N_2O production rate increased as the redox potential decreased. The same relationship between redox potential and the amount of N_2O accumulated was found in treatments with and without C_2H_2 blockage before the maximum N_2O accumulation. The amount of N_2O accumulated decreased after the maximum due to the less N_2O production when nitrate was limited, and more N_2O reduction activity when N_2O concentration was higher and N_2O reduction activity was not inhibited by C_2H_2.

It is technically incorrect to determine the critical redox potential for N_2O production from the occurrence of N_2O accumulation while N_2O reduction still functions. In such situation, N_2O could be reduced while it is produced in a denitrification process resulting in an insignificant accumulation. Unfortunately, in previous attempts to estimate the critical redox potential for N_2O production, no blockage of N_2O reduction was applied (Kralova et al., 1992; Smith et al., 1983). In this study, the critical redox potential to initiate N_2O production was estimated using widely accepted C_2H_2 inhibition technique. With inhibition by C_2H_2, all of the N_2O produced in denitrification was accumulated and could be detected by gas chromatography. The results showed that the critical redox potential to initiate N_2O production in denitrification was higher than previously reported results (Kralova et al., 1992; Smith et al., 1983). The redox potentials for N_2O production in denitrification to occur were all above +350 mV in the four tested soils (Figure 3.1). Nitrous oxide production in the Chinese rice soil likely occurred when the redox potential was higher than +400 mV, but unfortunately no

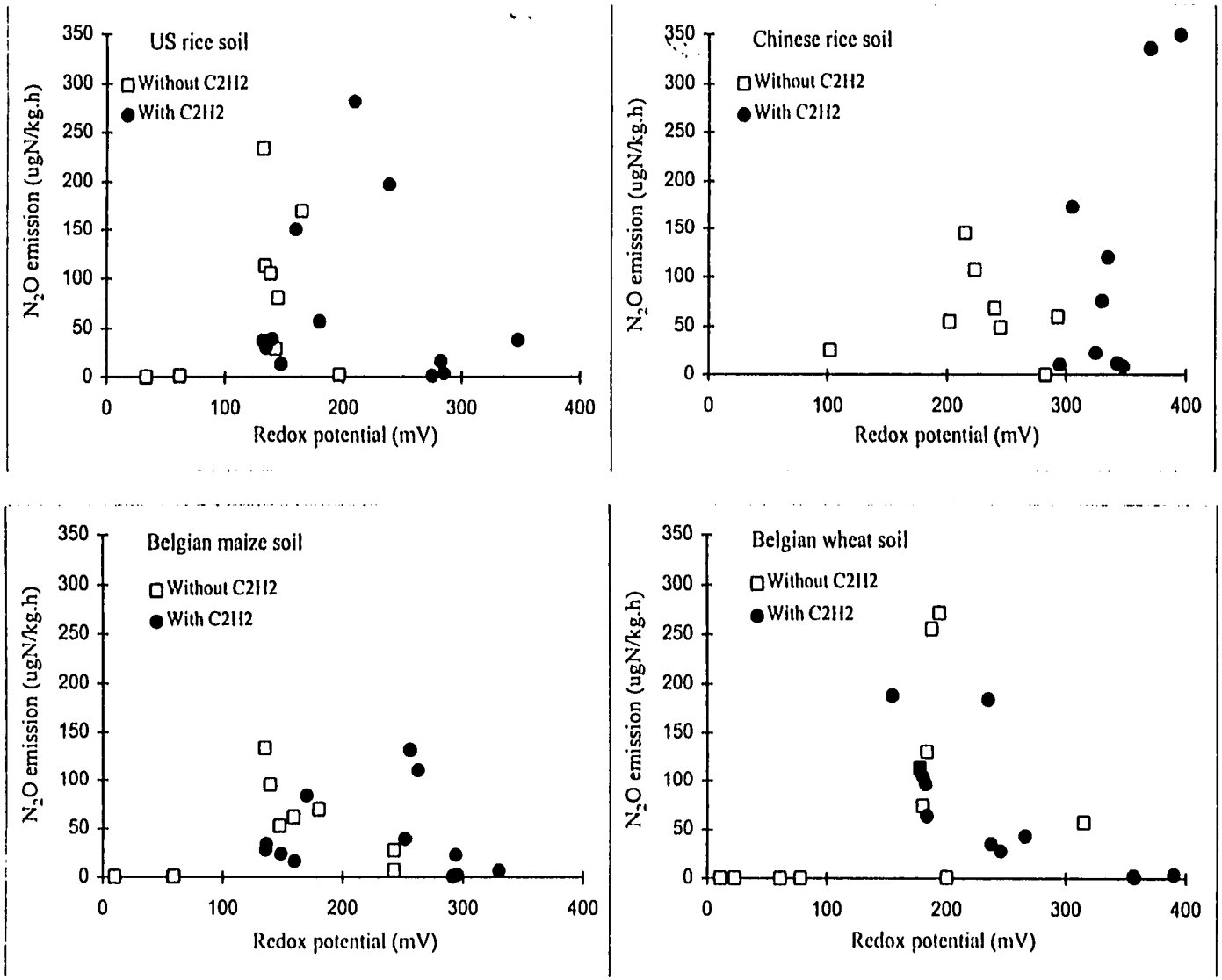


Figure 3.1 Nitrous oxide emissions at different soil redox potentials (from treatment A and B)

measurements were conducted above that level. Without C_2H_2 inhibition, the maximum N_2O accumulations occurred at lower redox conditions in comparison to that with C_2H_2 inhibition. The critical redox potentials for N_2O production estimated from the N_2O accumulation without C_2H_2 were located in the range of +200 to +300 mV, which were consistent with the early studies.

By definition, the critical redox potential to initiate denitrification should be estimated from the point where nitrate reduction occurs. The critical redox potential values for denitrification might be even higher than those of N_2O production with C_2H_2 inhibition in this study. Nitric oxide (NO) has been reported to be an obligatory intermediate product in denitrification (Ye et al., 1994), but it was not analyzed in this study, assuming that NO formed in denitrification will be immediately reduced. Some research indicated that the total N_2O accumulated in the presence of C_2H_2 might not be a good indicator of total denitrification (the amount of nitrate reduced). The amounts of N_2O and N_2 evolved accounted for less than 50 % of the observed decrease in nitrate-N (Kralova et al., 1992). The most possible mechanisms responsible for such observed discrepancy was the production of NO that was not analyzed. The presence of NO made denitrification study more complicated, because it could affect both N_2O production and reduction as demonstrated by Payne (1973). It is technically ideal to estimate the relation of denitrification and redox potential by the dissimilatory nitrate reduction instead of by the N_2O accumulation. It is profoundly important to distinguish the difference of N_2O accumulation with and without N_2O reduction inhibition.

Estimation of Critical Redox Potential for Nitrous Oxide Reduction

It has been generally believed for a long time that N_2O reduction can only occur after initiation of N_2O production in denitrification. However, some important facts probably have been ignored in this issue:

1) It is a process sequence that N_2 production comes later than N_2O production in a denitrification reaction. In a complete denitrification process N_2O always comes earlier than N_2, because N_2O functions as the substrate for the reaction of N_2O reduction to N_2;
2) Nitrous oxide reduction enzyme level is low in aerobic conditions, and it needs more time to be induced upon anoxia. In addition, N_2O reduction activity is commonly inhibited by other factors, such as O_2, nitrate, and low pH;
3) Nitrous oxide is actually a strong oxidant (further discussion can be found in Chapter V) with a standard redox potential of N_2O/N_2 higher than O_2/H_2O. It is thermodynamically more favorable that N_2O reduction proceeds early than nitrate reduction.

Production and reduction of N_2O during the denitrification process were found to be time dependent. The dissimilatory nitrate reductase develops rapidly, but the dissimilatory N_2O reductase only develops after a certain period of anaerobic conditions. Inhibition of nitrate and acidic conditions on N_2O reductase has been well recognized (Blackmer and Bremner, 1978a; Struwe and Kjøller, 1994). It is important to point out that it is the staggered synthesis of enzymes in response to anoxia and the inhibition of N_2O reductase by the presence of nitrate leads to an initial production of N_2O, and the temporal changes in N_2O and N_2 evolution during denitrification. After a certain period

of anaerobiosis, the accumulated N_2O was reduced to N_2, and finally the content of N_2O approached zero (Firestone and Tiedje, 1979; Letey et al., 1980b).

It has been taken for granted that the critical redox potential for N_2O reduction must be lower than that for N_2O production. In a previous study with the US rice soil, no additional nitrate was added and all the native nitrate would have been denitrified during the pre-incubation at -200 mV for approximately 14 days. The critical redox potential for N_2O reduction was found approximately to be +310 mV at pH 5, and +250 mV at pH 6, 7, and 8.5 (Smith et al., 1983). Sometimes the redox potentials where N_2O production and reduction initiated were not distinctly different. Letey et al. (1981) concluded in their study that the redox potential between +200 and +250 mV was critical for N_2O production and reduction. In this study, it was found that added N_2O could be reduced in all studied redox potential ranges when nitrate was not present or present at low concentration. It was estimated that the critical redox potential to initiate N_2O reduction could be up to +400 mV, or even higher (Figure 3.2). No definite relationship between N_2O reduction activity and soil redox potential was found, because redox potential is just one of the factors that affect the N_2O reduction activity. Other factors, such as pH, presence of NO, nitrate and organic matter content, might play a more important role in N_2O reduction activity.

The weaker the N_2O reduction activity in a soil, the less significant the effect of C_2H_2 inhibition on N_2O reduction to N_2. The weakest N_2O reduction activity in this study was found in the Belgian wheat soil, resulting in a significant overlap of N_2O accumulations with and without C_2H_2. The maximum N_2O accumulations with and without C_2H_2 in the other three soils were considerably separated due to the stronger N_2O

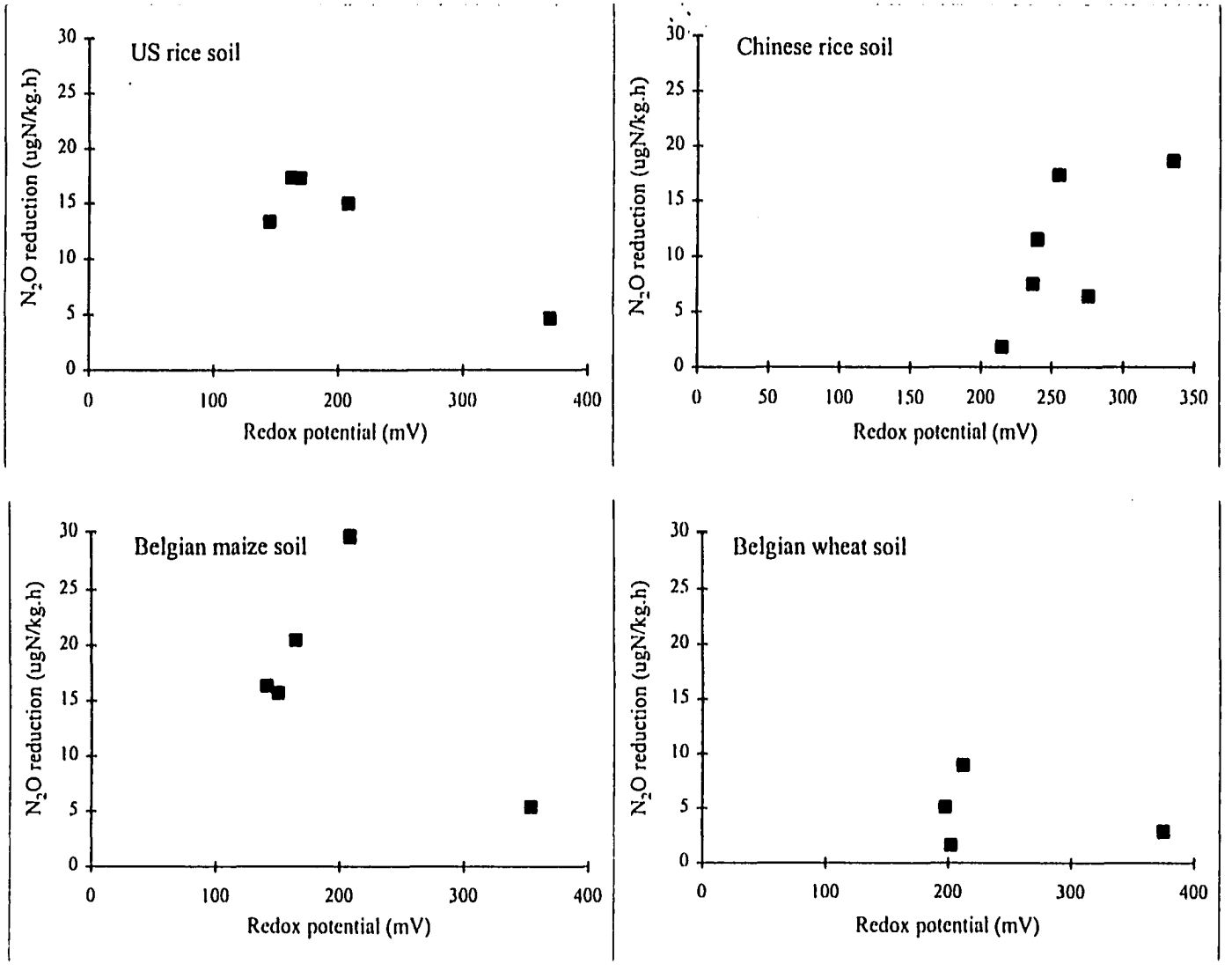


Figure 3.2 Nitrous oxide reductions at different soil redox potentials (from treatment C)

reduction activities. It was interesting to notice that the Chinese rice soil exhibited a strong N_2O reduction activity at higher redox potentials up to +350 mV, which helped to consume the N_2O produced at the same redox conditions. It can well explain the result that N_2O did not accumulate significantly at such redox potential range when N_2O reduction was not inhibited by C_2H_2 (Figure 3.1 and 3.2).

Nitrous oxide is an obligatory intermediate in the dissimilatory reduction of nitrate to N_2. Research by soil and atmospheric scientists has also suggested that increased N_2O emissions from flooded soils via denitrification of fertilizer and soil N contribute a major part of the present global N_2O flux (Letey et al., 1981). Denitrification is the only known biological mechanism to consume N_2O (Bremner et al., 1980; Hutchinson and Davidson, 1993). The results from this study provided some insight into N_2O production and reduction in relation to soil redox potential. It might help to make proper management to minimize N_2O emissions from soils, and even provide some possibility to make soils a significant atmospheric sink of N_2O, thus reducing the residence time of N_2O in the atmosphere.

CHAPTER IV NITROUS OXIDE AND METHANE EMISSIONS FROM DIFFERENT SOIL SUSPENSIONS: EFFECT OF SOIL REDOX STATUS

INTRODUCTION

Nitrous oxide (N_2O) and methane (CH_4) are two important greenhouse gases emitted mainly from biotic sources (Duxbury et al., 1993). Most N_2O is formed in O_2 deficient environments and is considered to come from denitrification, although it can also be produced during nitrification (Williams et al., 1992; Rice and Rogers, 1993). Methane is produced under low redox potential conditions by obligate anaerobes through either carbon dioxide (CO_2) reduction or transmethylation processes (Vogels et al., 1988). Methanogenesis and N_2O production are affected by many physical and biochemical factors, such as soil pH, redox potential, organic matter content, temperature, soil moisture content, etc. The content of soil oxidants (O_2, NO_3^-, Mn^{4-}, Fe^{3-}, SO_4^{2-} and CO_2) used as electron acceptors for organic matter degradation contributes significantly to these processes. The reduction of various oxidants in homogeneous soil suspensions occurs sequentially at corresponding soil redox potential values (Ponnamporuma, 1972). Flooded rice fields are considered one of the most important sources of atmospheric CH_4 and N_2O, because of the co-existence of both aerobic and anaerobic environments (Reddy et al., 1989). Methane production rate is usually high in flooded soils with high organic carbon content. These soils are net N_2O emitters as well if not constantly flooded, because of the availability of nitrate for denitrification being formed during temporary oxidizing conditions, enabling nitrification to take place (Byrnes et al., 1993). A reduced flooding duration increases the N_2O production, whereas continuous flooding maintains

anaerobic conditions and hence enhances CH_4 production (Neue, 1993). It is obvious that the factors affecting CH_4 and N_2O emission are complicated and internally related. A better understanding of this relationship is needed to be able to mitigate the emission of these important greenhouse gases through changes in agricultural practices.

The objectives of this laboratory study with four different soils were: (1) to investigate the soil redox potential range at which N_2O and CH_4 are produced, (2) to estimate the critical soil redox potential for initiation of CH_4 production, and (3) to study the relationship between CH_4 production and soil redox potential. The results should help to identify the redox potential range at which both gas emissions are at a minimum, and thereby to provide a basis for developing management strategies that will minimize the emissions of these greenhouse gases.

MATERIALS AND METHODS

Soils and Incubation Procedure

Four different soils listed in Appendix III were used in this study with application of the microcosm incubation technique described in Appendix IV. The soils were pre-incubated with no stirring and exposure to O_2 for one month in order to remove original nitrate, and to allow methanogens to become established. Then all four microcosms were stirred with a magnetic stirrer and purged with air for two days to oxidize the soil, so that the soils could experience the whole range of aerobic to anaerobic condition during the incubation. Four grams of dextrose (4.0 mg C g^{-1} soil) were added to each soil suspension as an energy source for the microorganisms, and potassium nitrate (KNO_3) was added to provide 50 μg N g^{-1} soil. The microcosms were sealed, and the soil

suspensions were continuously stirred by a magnetic stirrer during the incubation with no further O_2 supply.

Methane and Nitrous Oxide Measurement

During the incubation period, soil redox potentials in the microcosms decreased while various soil redox reactions were sequentially taking place. Whenever considerable redox potential changed, the microcosms were purged with pure nitrogen gas, and then were incubated for one day. The accumulated headspace gas was withdrawn in duplicate using a syringe, and was transferred into evacuated vials (10 ml Vacutainer, Becton Dickinson, New Jersey, U.S.A.). When CH_4 production rate started to decrease after a period of establishment of the strictly reducing conditions, the measurements were stopped. The same measurements were repeated twice, starting from purging the microcosms with air, in order to verify the results and to collect enough data, especially for N_2O emission because soil redox potential dropped quickly at the beginning of the incubation. Nitrous oxide and CH_4 were analyzed with a Tremetrics 9001 gas chromatography using an electron capture detector (ECD) for N_2O and a flame ionization detector (FID) for CH_4. The emission rates of N_2O and CH_4 were calculated as the amount of gas accumulation divided by the accumulation time and the amount of soil used. Redox potential values and N_2O and CH_4 emission rates were reported as a mean of duplicate measurements. The significance of the relationship between redox potentials and CH_4 emissions was determined statistically by the student t-test.

RESULTS AND DISCUSSION

A well-oxidized soil has a redox potential range of +400 to +700 mV. Flooded soils may reach redox potential values of lower than -300 mV due to the absence of O_2

and the activity of facultative and obligate anaerobic bacteria (Patrick and Mahapatra, 1968). The rate of change in the soil redox potential was soil specific. It depended on the original content of soil oxidants and reductants, as well as on the difference in population and types of soil microbial communities, which contributed to the soil redox reactions and each reaction rate. The soil redox potential values measured in this study were generally in the range +400 to –300 mV. For the two rice soil suspensions, about one month was required to undergo such a redox potential change, while about two months were required for the two upland soil suspensions. The two upland soils required a longer time to be reduced because, due to the original aerobic environment, they likely had more oxidized components (such as iron oxides) than flooded rice soils. Some oxidized compounds in rice soils that have undergone cycles of flooding and draining tend to be converted to their more mobile counterparts (i. e. Fe^{3+} to Fe^{2+} and SO_4^{2-} to H_2S) that move out of the system following flooding. The difference in microbial community between upland soils and rice soils may also account for the different time required to complete the above redox potential range. At the end of the experiment, the pH values of the four soil suspensions reached a narrow range with 6.7 for US rice soil, 7.2 for the Chinese rice soil, 6.5 for the Belgian maize soil, and 6.9 for the Belgian wheat soil, respectively.

Nitrous Oxide Emission

Denitrification was considered to be the major source of N_2O production in this study as anaerobic conditions prevailed. The critical redox potential for denitrification found in a previous study using US rice soil was approximately +350 mV (Patrick and Jugsujinda, 1992). Nitrous oxide emissions from the four soils over a range of redox potential conditions were shown in Figure 4.1. For all soils, there was a narrow range of

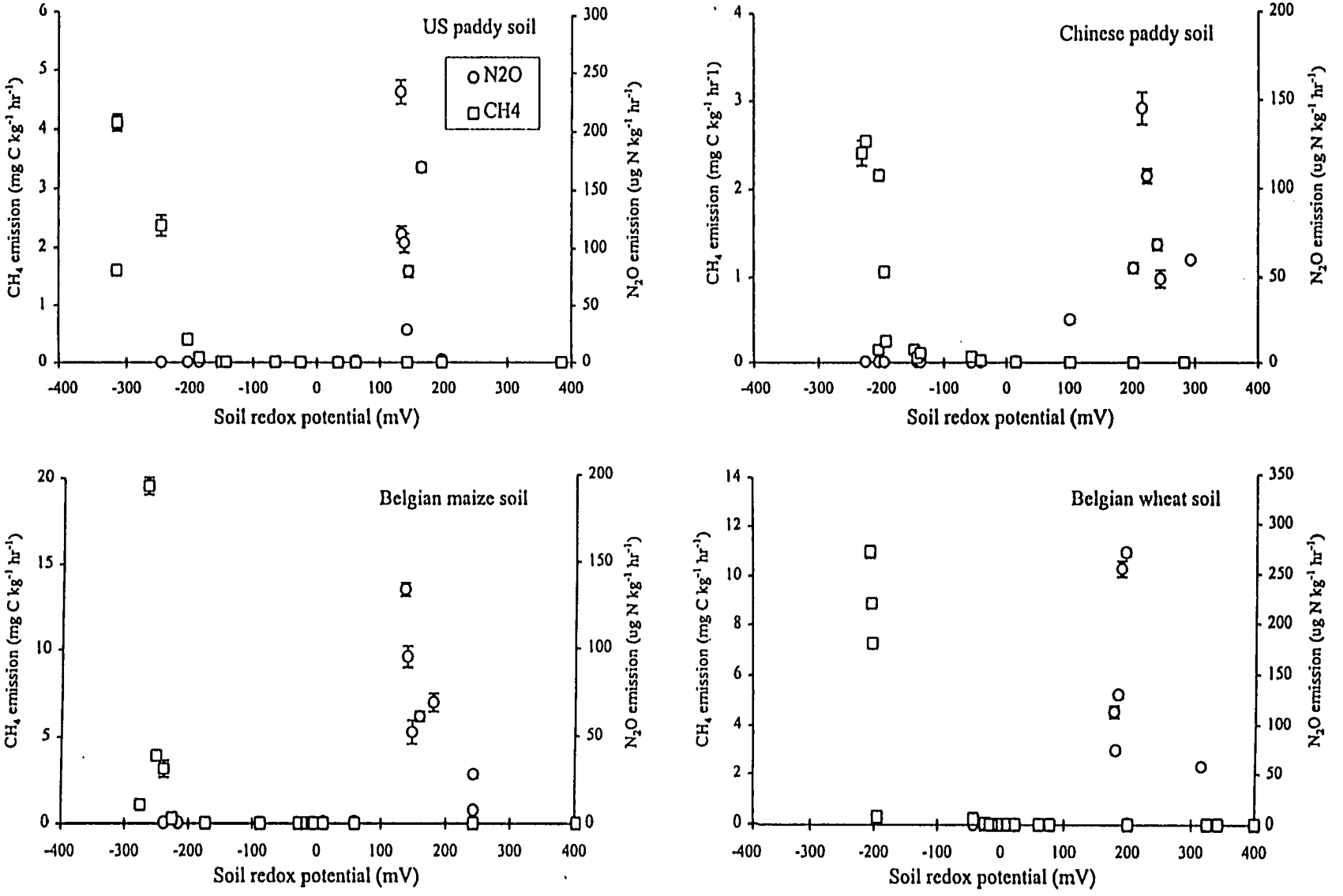


Figure 4.1 Nitrous oxide and methane emissions at different soil redox potentials
Points represent the means ± standard deviations of two replicate gas sampling.

redox potential where N_2O accumulated significantly. The results show a significant N_2O accumulation in the soil redox potential range between +120 to +250 mV, while the maximum emission rate was between 140 to 280 μg N kg^{-1} hr^{-1}. Little N_2O emission occurred at redox potential values higher than +250 mV or lower than +120 mV. The results also indicate the influence of different soils on N_2O emission. The maximum N_2O emission varied two fold for the four soils, with the maize field soil showing 134 μg N kg^{-1} hr^{-1} at a redox potential of +135 mV, the wheat field soil 272 μg N kg^{-1} hr^{-1} at +194 mV, the US rice soil 234 μg N kg^{-1} hr^{-1} at +133 mV, and the Chinese rice soil 145 μg N kg^{-1} hr^{-1} at 215 mV. It is important to understand that the N_2O emission is the balance of N_2O formation and further reduction, both greatly depend on the origin of the soil, nitrate availability, pH and redox potential status. There was no clear relationship between the maximum N_2O accumulation and soil redox status. It should also be pointed out that the measurement might have not covered the actual maximum N_2O accumulation because of the fast change of soil redox potential at the beginning of the incubation.

Methane Emission

Methane emission from the four soils under different redox potential conditions is also shown in Figure 4.1. Methane production occurred after extended anaerobic incubation in all four soils. The emission of CH_4 from soils is the net result between CH_4 production and oxidation. The CH_4 oxidation activity has been found mainly under aerobic conditions. However, there is evidence that CH_4 can be oxidized under anaerobic conditions, but the oxidation rate is comparatively low (Panganiban et al., 1979; Reeburgh, 1980; Miura et al., 1992). Thus, the CH_4 emissions from the microcosms

could be regarded as soil CH_4 production potentials. Methane production in the two upland soils, 19.4 mg C kg^{-1} hr^{-1} at -265 mV in the maize soil and 10.9 mg C kg^{-1} hr^{-1} at -210 mV in the wheat soil, was higher than the CH_4 production in the two rice soils, 4.15 (at -315 mV) in the US soil and 2.53 (at -225 mV) mg C kg^{-1} hr^{-1} in the Chinese soil. A significant linear relationship was found between the natural logarithm of the CH_4 emissions and the soil redox potentials in all four soil suspensions (Table 4.1). This corresponds to the result obtained previously on the same US rice soil (Wang et al., 1992) and indicates that CH_4 production in the soils was of biological origin, and that the CH_4 production activity increased exponentially when the soil redox potential dropped below a critical point.

Significant CH_4 production occurs under strictly anaerobic conditions. The critical soil redox potential for CH_4 production from the same US rice soil has been reported to be -150 to -160 mV (Wang et al., 1992). The critical redox potential reported depended on both the soil and the method used to estimate the critical point at which CH_4 production commenced. In this study, the critical redox potential for CH_4 production was considered as a certain soil redox potential point below which the CH_4 emission rate reached a positive value. The critical redox potential is technically impossible to determine by direct examination of the logarithm plots of the emission values against the redox potential values. Therefore in this study, this critical point was estimated by linear regression of the CH_4 emissions against the soil redox potential and extrapolating the linear curve to the point where the CH_4 emission was zero. The linear relationship between CH_4 production and soil redox potential was also found to be significant in these four soils, suggesting that the estimation of the critical redox potential for CH_4 production

by this method is acceptable. The results show that the critical soil redox potential for CH_4 production to occur was in the range of -150 to -210 mV (Table 4.1). The slope in the linear regression between the soil redox potential values and the CH_4 production represents the increase of the CH_4 production rate with decreasing soil redox potential. The lower the critical soil redox potential for CH_4 production, the greater the increase of CH_4 production with a decrease soil redox potential. A significant exponential relationship between the critical redox potentials for CH_4 production and the maximum CH_4 production rates recorded was also found in the four soils (Figure 4.2). It suggested that a soil with a lower critical redox potential for CH_4 production has a higher potential for CH_4 production. The exponential relationship between soil redox potential and CH_4 production likely exists not only in a single soil, but also among different soils. The result indicates that it is essential to keep a relatively higher soil redox potential in any soils whenever CH_4 production control is required.

The estimation of the critical redox potentials for CH_4 production in this study was based on vigorous CH_4 production under different redox conditions. Because of the inhibition of methanogenesis (both methanogen population and activities) by other oxidants, significant CH_4 production can only occur when such inhibition is taken away as indicated by a critical low point of redox potential (Cicerone and Oremland, 1988). Limited CH_4 production was observed during the initial phase of anoxia in rice soil slurries despite a high redox potential and the presence of oxidants (Roy et al., 1997). The presence of methanogens and the evolution of H_2 at the beginning of soil submergence make early initiation of methanogenesis thermodynamically possible, but it is only theoretically important and it does not provide information on the quantity of CH_4

Table 4.1 Relationship between redox potential and CH_4 production and estimation of the critical redox potential for CH_4 production

Soil type	Exponential regression		Linear regression		
	Equation	R^2	Equation	R^2	Critical Eh
US rice soil	$Eh = -23.4 \times C_2 - 246.2$	0.85 (n = 5) **	$Eh = -33.8 \times C_1 - 171.8$	0.97 ***	-170
Chinese rice soil	$Eh = -23.2 \times C_2 - 201.5$	0.90 (n=7)***	$Eh = -30.4 \times C_1 - 149.3$	0.90 ***	-150
Belgian maize soil	$Eh = -11.4 \times C_2 - 231.9$	0.68 (n=5)***	$Eh = -3.0 \times C_1 - 214.3$	0.68 *	-215
Belgian wheat soil	$Eh = -3.7 \times C_2 - 199.4$	0.95 (n=4)**	$Eh = -6.4 \times C_1 - 194.6$	0.95 **	-195

Where

Eh = soil redox potential (mV);

C_1=CH_4 production (mg C kg^{-1} hr^{-1}), and C_2=ln C_1;

n = number of samples;

* = 90 % confidence level,

** = 95 % confidence level, and

*** = 99 % confidence level.

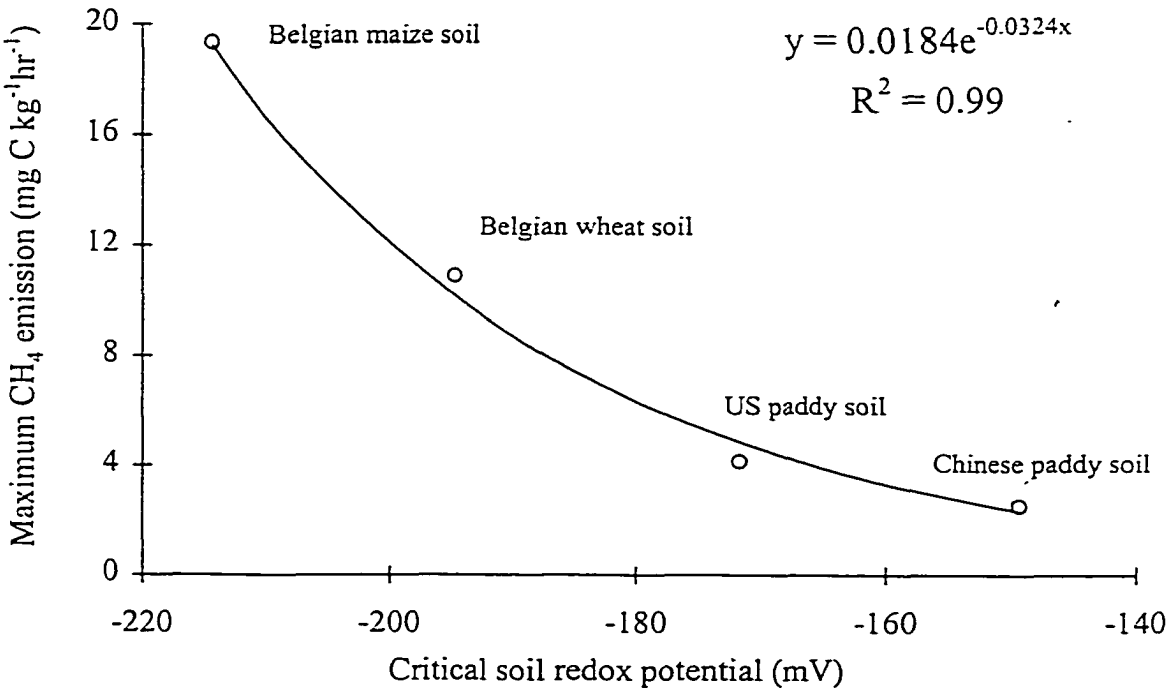


Figure 4.2 Relationship between the critical redox potentials for CH_4 production and the maximum CH_4 emission in different soils

produced. When CH_4 concentrations are plotted on a linear scale, CH_4 production occurred mostly after the complete reduction of SO_4^{2-} by sulfate-reducing bacteria.

The effect of soil redox status on CH_4 and N_2O emissions from the US rice soil was previously studied under controlled redox potential range from +500 to -250 mV (Masscheleyn et al., 1993). However, carbon and nitrate were added at each redox potential level, and the redox potential range where both N_2O and CH_4 emissions were low was not clearly identified because nitrate was actually not present when soil redox potential was maintained at value lower than +100 mV. A different experimental approach was used that the redox potential in the soil suspension was measured but not controlled. Nitrous oxide and CH_4 emissions from the soil suspensions were quantified by frequent sampling during the natural decrease of soil redox potential by microbial aerobic and anaerobic respiration. This is more similar to the actual rice field where soils are continuously flooded.

Redox Potential Range for Minimum Methane and Nitrous Oxide Emission

Nitrous oxide and CH_4 emissions were found to occur at different soil redox potential conditions. There was a distinct soil redox potential range where neither N_2O nor CH_4 emissions were significant (Figure 4.1). This soil redox potential range was slightly different among the four tested soils, because the critical redox potentials for either CH_4 production or N_2O accumulation were different. The range of minimum accumulation of both CH_4 and N_2O was generally situated between +120 to –170 mV. Nitrous oxide reduction was stronger than its production in such a redox potential range, while no significant CH_4 production occurred. These results are important for field practices with regard to greenhouse gases management. On one hand, it indicates the risk

of stimulating N_2O production in trying to diminish CH_4 production by increasing the soil redox potential (e.g. by soil drainage or withholding organic matter from the soil). It also demonstrates the difficulty of controlling N_2O emission by keeping the soil reduced enough only to favor N_2 production during the denitrification process, but not reduced enough to produce CH_4. On the other hand, such a wide redox potential range where neither of these two gases is accumulated should make it possible to minimize both N_2O and CH_4 emissions from wetland ecosystems by carefully regulating the water supply and organic matter amendments. A long-term study in a Chinese rice field showed that there was a favorable soil redox potential condition (0 to +100 mV at 10 cm depth of the soil) where both N_2O and CH_4 emissions were low (Hou et al., in press). It will be difficult to keep the soil profile in such a favorable redox potential range during the whole rice growing season. However, it is possible to significantly reduce CH_4 emission by carefully managing irrigation and drainage practice without inducing significant N_2O emission, especially during period of vigorous CH_4 flux. Organic matter, as an electron donor, is an important factor in regulating the N_2O/N_2 ratio in denitrification, because it will favor N_2O reduction to N_2 by releasing the electron competition between N_2O reduction and nitrate reduction. When electrons are abundant (i. e. soil is more reduced), denitrification tends go to completion with N_2 as end product (Murakami et al., 1987).

Implications of This Study

Rice fields are a favorable environment for both N_2O production and methanogenesis because of their changing redox potential condition. An inverse relationship of N_2O and CH_4 emissions has been found in a long-term Chinese rice field study (Chen et al., 1997). Water and nutrient management, effect of rice cultivars and

other agronomic practice have been tested to mitigate CH_4 production and emission from rice fields, among them water management is the most effective (Yagi et al., 1990; Sass et al., 1992). Possibilities for reducing CH_4 emissions were evaluated in the National Inventories of the CH_4 and N_2O Workshop (Khalil, 1993). One of the principles that must be followed in developing a practice to reduce CH_4 emissions from flooded rice soils is that the mitigation practice should not increase the emissions of other greenhouse gases, particularly N_2O. The results of this laboratory experiment provide further insight into the effect of soil redox potential on N_2O and CH_4 production and emission. It points to the importance of N_2O reduction, through which the N_2O emission is regulated in a narrow soil redox potential range. It also shows the risk for CH_4 production when the soil redox potential status drops to a lower level. In this study, a wide soil redox potential range was found where both N_2O and CH_4 emissions were low, which provides an opportunity to minimize the emissions of these two important greenhouse gases in the field.

CHAPTER V IMPLICATION OF NITROUS OXIDE, A STRONG OXIDANT, ON SOIL OXIDATION-REDUCTION CHEMISTRY

INTRODUCTION

Most soil oxidation-reduction reactions occur in a redox potential range where water (H_2O) is stable, and the reactions are sequentially initiated following the decrease of redox potential as predicted in theory of redox chemistry (Table 5.1). When O_2 availability becomes limited, denitrification starts resulting in the disappearance of nitrate and temporary accumulation of N_2O. Denitrification has been intensively studied for its removal of nitrate decades ago, and for its emission of N_2O in recent years. Nitrous oxide is one of the chemically reactive greenhouse gases in the atmosphere responsible for the catalytic destruction of stratospheric ozone (Crutzen, 1981; Dickinson and Cicerone, 1986; Weiss, 1981). In most natural environments, N_2O is present in trace amounts, and in the atmosphere, N_2O concentration is currently about 310 ppbv. However, higher concentrations of N_2O can be found in soil pore water supersaturated with N_2O (Amundson and Davidson, 1990; Minami, 1987). There are still many facts ignored or unrecognized regarding N_2O production in denitrification and its implications for other soil oxidation-reduction processes. The standard redox potential of the N_2O/N_2 pair is 1770 mV, even higher than that of O_2/H_2O (1229 mV), which suggests that N_2O is a potentially strong oxidant. In this study, the goal was to provide some evidence of N_2O actually being an oxidant, and its effect on soil redox potential and other soil oxidation-reduction reactions. The temporal presence of nitric oxide (NO) in denitrification makes some experimental results difficult to interpret. A discussion regarding the implication of

Table 5.1 Oxidation-reduction potential of some important soil reactions

Reaction	Standard Eh (mV)	Eh (mV) at different pH		
		pH=6	pH=7	pH=8
Typical reactions				
$O_2 + 4H^+ + 4e^- = 2H_2O$	1229	874	815	755
$2NO_3^- + 12H^+ + 10e^- = N_2 + 6H_2O$	1240	815	744	674
$MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$	1230	520	410	283
$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$	1060	-6	-183	-361
$SO_4^{2-} + 10H^+ + 8e^- = H_2S + 4H_2O$	300	-144	-218	-292
$CO_2 + 8H^+ + 8e^- = CH_4 + 2H_2O$	170	-185	-244	-304
$2H^+ + 2e^- = H_2$	0	-355	-414	-474
Other reactions				
$N_2O + 4H^+ + 4e^- = 2\ N_2 + H_2O$	1770	1415	1356	1296
$NO + 4H^+ + 4e^- = N_2O$	1590	1235	1176	1116
$2NO_3^- + 12H^+ + 10e^- = NH_4^+ + 6H_2O$	880	436	362	288

Calculation of Eh is according to Nernst equation

$$Eh = E^\circ - 2.303RT/nF \log [Reductant]/[Oxidant]$$

E°, standard redox potentials were cited from (Lide, 1991. Handbook of Chemistry and Physics)

NO on soil redox chemistry is beyond the scope of this dissertation since it is not determined, but a brief discussion is included.

Evidence of Nitrous Oxide as an Oxidant

In an attempt to oxidize a reduced medium with 0.0025 % (wt/vol) of cysteine hydrochloride and $Na_2S.9H_2O$, Jenneman et al. (1986) found that the addition of 380 nM of N_2O completely oxidized the medium. However, the addition of up to 72 mM sodium nitrate or 59 mM sodium nitrite did not oxidize the medium. When N_2O levels were measured by gas chromatography, it was observed that N_2O accumulation immediately preceded the oxidation of the medium and N_2O levels declined before the re-reduction of the medium. They also demonstrated that NO probably was a more effective oxidant that about 10 nM of NO could completely oxidize the same medium. In fact, the above results could be expected by comparing the redox potential of each reaction. The redox potentials of NO_3^-/NO_2^- and NO_3^-/N_2 pairs are only +433 and +744 mV at pH 7, respectively. Whereas N_2O/N_2 and NO/N_2O redox couples are +1356 and +1176 mV, respectively (Thauer et al., 1977), which indicate that N_2O and NO are stronger oxidants than nitrate and nitrite.

One of the applications of using N_2O as an oxidant can be found in flame atomic absorption spectrophotometry (Reynolds et al., 1970; EPA, 1983). A flame is required in this technique to convert elements in a liquid sample into free atoms for detection. The most commonly used combustible gas mixtures are acetylene with air, and acetylene with N_2O. Whenever higher temperature is needed in some particular applications, instrument grade N_2O is used as a combustion supporting gas (an oxidant). The reaction of N_2O with

Table 5.2 Comparison of energy yield in reactions with O_2 and N_2O as oxidants

Reaction	Energy yield (kJ mol^{-1})	
	with O_2	with N_2O
Combustion of acetylene in flame atomic absorption	1267.4	1785.9
Respiration using glucose	2867.5	4113.1
Oxidation of soil reductants		
Mn^{2-} to MnO_2	79.6	183.3
Fe^{2-} to $Fe(OH)_3$	67.3	119.1
S^{2-} to SO_4^{2-}	795.7	1210.6
CH_4 to CO_2	817.1	1232.0

Data of Gibbs free energy were cited from Handbook of Chemistry and Physics (Lide, 1991)

acetylene yields more energy than an acetylene and air mixture (Table 5.2), and consequently generates higher temperature in the flame.

Emission of N_2O from industrial plants producing adipic acid (for manufacture of nylon fibers and plastics), is a major anthropogenic point source of N_2O (see Appendix II). To produce adipic acid, benzene is hydrogenated to form cyclohexane, then cyclohexane is oxidized to form a mixture of cyclohexanol and cyclohexanone that are reacted with nitric acid to make adipic acid. Nitrous oxide is an intrinsic byproduct of this chemical reaction (Thiemens and Trogler, 1991). Emission of N_2O in the adipic acid industry currently accounts for about 5-8 % of the worldwide anthropogenic N_2O emissions. Technology options to reduce its emission include decomposition and reduction (Reimer et al., 1994). Nitrous oxide serves as an oxidant in the reactions of the reduction process:

$$N_2O + CH_4 \longrightarrow CO_2 + H_2O + N_2$$

$$N_2O + \text{Benzene} \longrightarrow \text{Phenol (Benzene-OH)} + N_2$$

In the second reaction, N_2O is recycled and economically more profitable in the adipic acid production.

Effect of Nitrous Oxide on Soil Redox Potential

To test the hypothesis that N_2O can cause an increase of soil redox potential, the two rice soils described in Appendix III were used in this experiment with the application of microcosm incubation technique as described in Appendix IV. The two rice soils were pre-incubated with addition of 4 g dextrose to each microcosm until strongly reducing conditions (about -200 mV) were established. To compare the effect of N_2O and O_2 on soil redox potential, N_2O and O_2 were introduced into the reducing soil suspensions

respectively. Oxygen was added by injection of 60 ml air (21% O_2) into each microcosm, and changes of redox potential in the soil suspensions were monitored. When the similar redox potential levels resumed, 60 ml N_2O (mixture of 98 % N_2O with 2 % N_2) was injected into each microcosm, and both soil redox potentials and N_2O concentrations in the microcosms were measured. Oxygen and N_2O was not provided at the same amount, but this preliminary study illustrated their different effects on soil redox potential. The addition of air resulted in an increase of soil redox potential by about 30 mV in the US rice soil, and about 20 mV in the Chinese rice soil (Figure 5.1 and 5.2). The effect of O_2 was temporal because it was consumed quickly by soil microbial respiration activities. It took about 5 days for the US rice soil to recover to the initial redox potential, while it took about 10 days for the Chinese rice soil. Addition of N_2O caused an increase of the soil redox potential of about 50 mV in the US rice soil, and about 100 mV in the Chinese rice soil. The increase of soil redox potential by addition of N_2O and O_2 was apparently soil dependent. It took a much longer time to recover the redox potential increase by the addition of N_2O compared to the addition of air. There are only limited number of microbes that can use N_2O for respiration activities, which resulted in the reduction of N_2O being a slow process. The presence of N_2O for a longer time might provide more chance for N_2O to affect other soil oxidation and reduction reactions.

The increase of soil redox potential caused by addition of an oxidant and the time needed to recover from such an increase also depend on the possible shift of oxidation and reduction reactions in the soils. In a closed incubation system, though conversions between oxidized and reduced forms are occurring at different redox conditions, there is no escape of substance out of the system. In anaerobic conditions some reductants, such

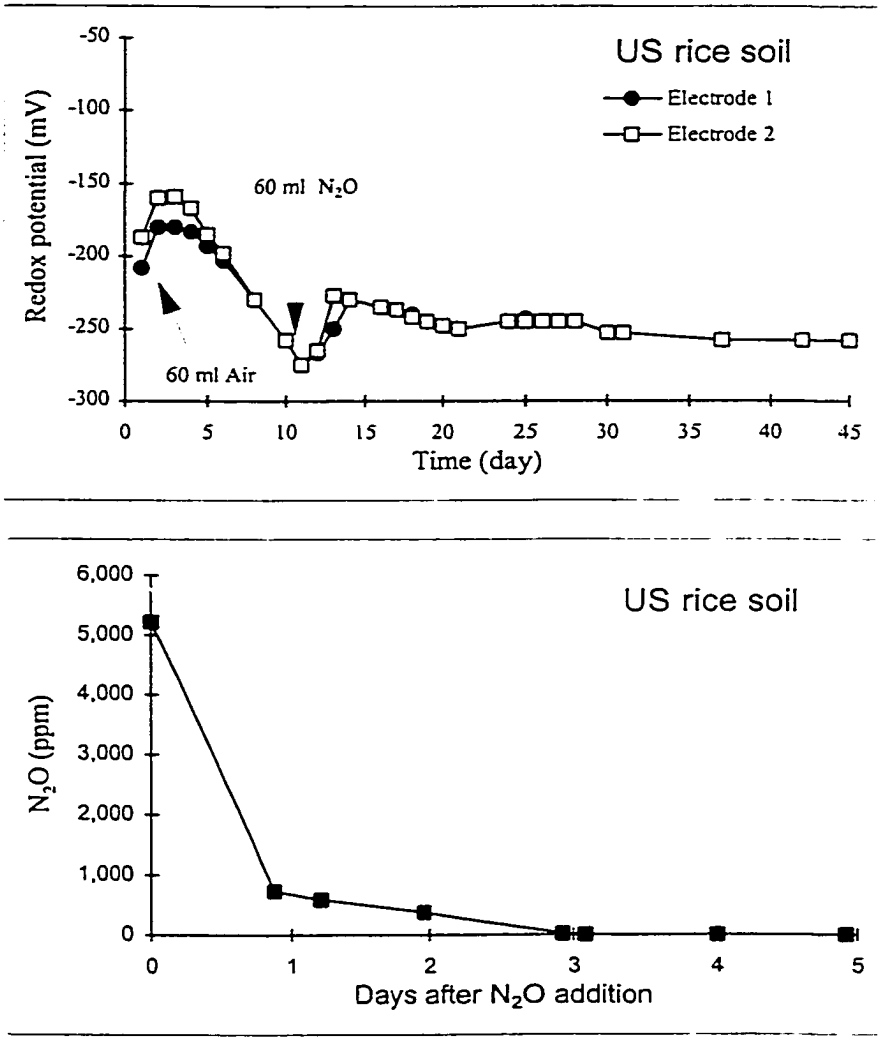


Figure 5.1 Comparison of the effect of O_2 and N_2O addition on redox potential in the US rice soil, and change of N_2O concentration following N_2O addition

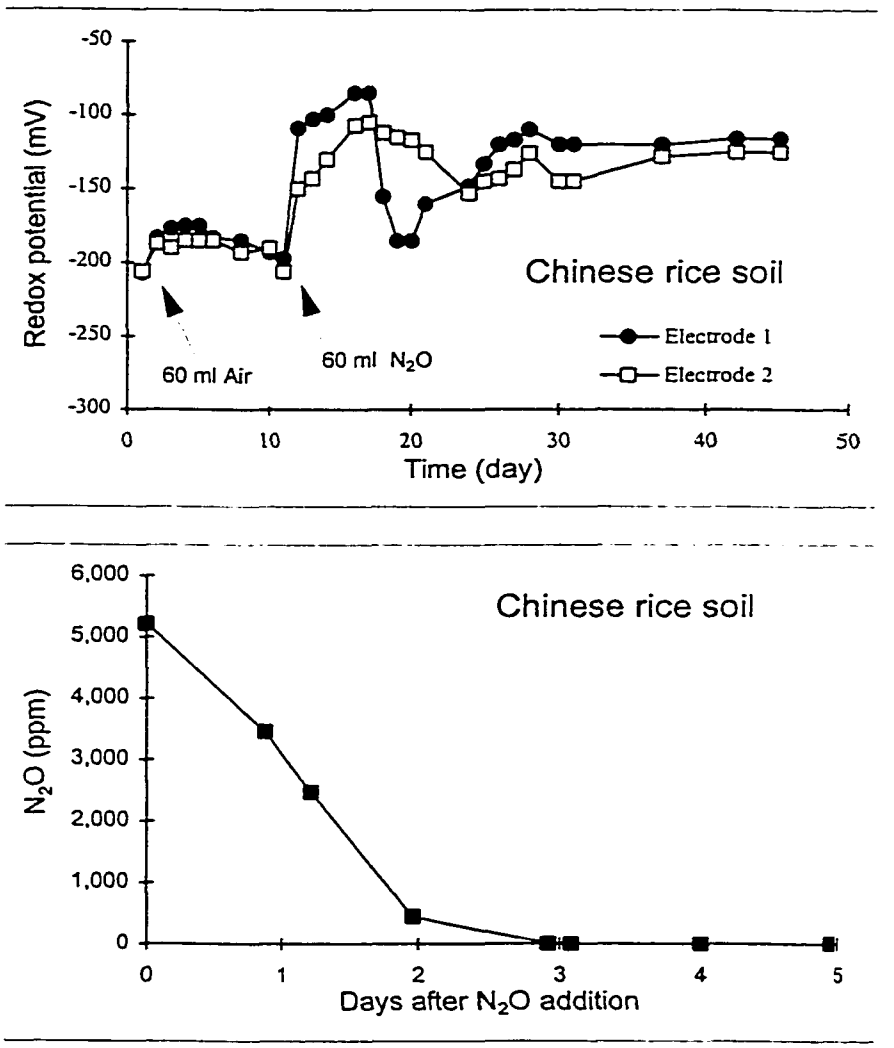


Figure 5.2 Comparison of the effect of O_2 and N_2O addition on redox potential in the Chinese rice soil, and change of N_2O concentration following N_2O addition

as Fe^{2+} and Mn^{2+}, are available and ready to be re-oxidized, for example by O_2 and N_2O, to their oxidized counterparts. This mechanism will cause an indirect increase of soil redox potential, which can help to explain the different responses of soil when O_2 or N_2O is added, and different time needed to recover the redox potential change. This is also probably the reason why the soil redox potential in the soil suspensions remained high when the added N_2O had almost been depleted (Figure 5.1 and 5.2). Same mechanism may also apply to the situation when O_2 was added, but change of O_2 concentration was not monitored in this study.

In actual rice fields, the presence of N_2O from denitrification may also contribute to the oxidation of CH_4 and H_2S before they evolve from the soils to the atmosphere. Anaerobic oxidation of CH_4 has been recognized and is considered to be an important mechanism of CH_4 consumption in soils (Murase and Kimura, 1994). It is important to recognize that all of the major soil oxidation-reduction reactions associated with O_2 can occur associated with N_2O in theory. The reactions associated with N_2O are eventually thermodynamically favorable because of more energy yield (Table 5.2). It is because the majority of soil microbes can utilize O_2 for respiration making oxidation reactions with O_2 dominant in nature. The limited number of microbes that can use N_2O as an electron acceptor and the common inhibition of N_2O reduction activity make the oxidation effect of N_2O less important and usually be ignored.

Implication of Nitrous Oxide on Soil Anaerobic Processes

Increase of soil redox potential can effectively inhibit some soil anaerobic reactions that require redox potentials below certain critical values. Biological sulfide production does not occur when the redox potential is above -100 to -150 mV (Postgate,

1979; Connell and Patrick, 1968). The critical soil redox potential for initiation of CH_4 production in the US rice soil was found to be about -150 mV (Masscheleyn et al., 1993; Wang et al., 1993).

Inhibition of sulfide production by nitrate addition has been reported. Early observation is that the addition of 1 gram of nitrate per liter to sewage sludge inhibited sulfide production for at least 29 days (Allen, 1949). This inhibition was attributed to the increase in redox potential caused by nitrate at that time. Poduska and Anderson (1981) found that nitrate addition controlled sulfide production in a waste water lagoon so long as enough nitrate was added initially to raise the redox potential of the lagoon above 300 mV. They also observed that once the redox potential was above that value, it was easily maintained at that level with little or no additional nitrate. The decrease in soluble organic matter was considered to be the major cause of the increase of soil redox potential in their study. In another study, nitrate, nitrite, and N_2O were detected during periods where sulfide production was inhibited, whereas nitrate, nitrite, and N_2O were below detectable levels at the time sulfide production began (Gary et al., 1986). The inhibition of sulfide production was due to the increase in the redox potential of the environment as a result of the action of nitrate-using bacteria. The cause of the increased redox potential was highly attributed to the accumulation of N_2O, or possibly of NO. In addition, the oxidation of the medium was associated with the accumulation and persistence of N_2O. This argument was supported by the observation that the addition of low levels of either N_2O or NO oxidized the reduced medium or diluted sewage sludge. The re-reduction of the medium was found to be associated with the decrease in N_2O concentration (Jenneman, et al., 1986). It may help to explain the lack of sulfide production observed

by Balderston and Sieburth (1976) in their aqua-culture system and the unexpected high redox potential (+222 mV) observed by Sørensen (1978a) in marine sediments amended with nitrate. It may also explain why addition of nitrate to anaerobic environment can cause inhibition of methanogenesis in some cases, as well as why strict anaerobes such as sulfate-reducing bacteria that respire nitrate reduce it to ammonium and not to N_2O or N_2 (Caskey and Tiedje, 1979; Keith and Herbert, 1983; McCready et al., 1983).

The inhibition of anaerobic reactions by the presence of N_2O can be prolonged, because consumption of N_2O is a slow process. The addition of a high concentration of nitrate leads to the buildup of N_2O, which raised the redox potential, resulting in the inhibition of sulfide production. Gary et al. (1986) concluded in their study that the prolonged oxidation of the medium was the result of N_2O production rather than O_2 contamination. Electron donor concentration was limited in their study with consequence of an even more slow N_2O destruction, which contributed to the prolonged oxidation of the medium. Sulfide production was not observed in bottles containing N_2O even after prolonged incubation. Kucera et al. (1983) found that the buildup of nitrite and N_2O can inhibit nitrate reductase by channeling electrons through nitrite and N_2O reductase. This may explain why nitrate was present after 5 months of incubation in the bottles, since large amounts of N_2O were also present, which could have inhibited the further reduction of nitrate.

Both experimental and theoretical evidence supports the argument that the biological production of N_2O can cause an oxidation of a reduced medium and an inhibition of other anaerobic reactions. However, NO production was possibly also important in this regard. Thus, there are two different reasons why sulfide production can

be inhibited for prolonged periods by nitrate addition. The first reason is the increase of soil redox potential by the presence of N_2O or NO or both, which results in an oxidized environment. The buildup of N_2O or NO will occur in environments with high denitrification capacities and in instances where the ratio of electron donor to electron acceptor (nitrate) is low. The second reason is that the level of sulfate reducing bacteria may be decreased during the prolonged exposure to an oxidizing environment. High concentration of N_2O appears to have a cyto-toxic effect on sulfate-reducing populations (Thom and Marquis, 1984), and NO is known to be bacteriostatic to certain bacteria (Mancinelli and McKay, 1983).

Dissimilatory nitrate reduction rather than denitrification is the predominant pathway for nitrate use when electron donor concentrations are high (King and Nedwell, 1985; Nedwell, 1982; Sørensen, 1978b; Tiedje et al., 1982). Nitrate disappeared in a fast rate with ammonium as an end product (Table 5.1). There is no buildup of N_2O in this situation. This is probably the reason why the inhibition of nitrate on sulfide and CH_4 production was not significant in some cases.

CHAPTER VI METHANOGENESIS AND DENITRIFICATION IN A STRATIFIED RICE SOIL

INTRODUCTION

Flooded rice fields are considered as one of the most important sources of atmospheric CH_4 and N_2O. Most of the N_2O produced in an "anaerobic" environment is considered a product of denitrification, although it can be produced during nitrification as well (Williams et al., 1992; Rice and Rogers, 1993). Methane is produced when soil redox potential decreases below a critical point by strictly true anaerobic microorganisms through either CO_2 reduction or transmethylation processes. Methanogenesis, nitrification and denitrification are soil microbiological processes affected by many physical and biochemical factors, such as soil pH, redox potential, temperature, available substrate for each process, and soil microbial communities and populations, etc. The content of soil oxidants (O_2, NO_3^-, Mn^{4-}, Fe^{3-}, SO_4^{2-} and CO_2) used as electron acceptors for organic matter degradation contribute significantly to these processes. The reduction of various oxidants in homogeneous soil suspension occurs sequentially at corresponding soil redox potentials (Ponnamporuma, 1972; Patrick and DeLaune, 1977). Both CH_4 and N_2O production are functions of soil redox potential and microbiological processes when corresponding substrate for CH_4 or N_2O production is not limited. Methane production rate is ordinarily high in flooded soils (usually reflected by low soil redox potential) with high organic carbon content. These soils are producers of N_2O as well if not constantly flooded because of the availability of mineral-N and the temporary oxidized condition that enables nitrification to take place (Byrnes, 1993). Reduced flooding duration increases N_2O production, whereas continuously flooded soils

maintains anaerobic conditions and hence enhance CH_4 production (Neue, 1993). It is obvious that the factors affecting CH_4 and N_2O emissions are complicated and internally related. The previous study was done in microcosms where soil was in homogenous condition (see Chapter IV). The results indicated that CH_4 and N_2O were produced and emitted at different soil redox potentials. The actual soil in the field may form a redox potential gradient along the soil profile during a prolonged flooded condition, which might provide a basis for CH_4 and N_2O production at different layers of a soil profile. In this experiment, an artificially packed soil core was used to determine in which layer CH_4 and N_2O will be produced and emitted, and the factors affecting the emission rates. The results may represent some actual situation in the field where plants are not involved.

Materials and Methods

Sample Soil and Stratification

Soil cores were artificially made by packing the mixed US rice soil (listed in Appendix III) into transparent plastic tubes (20 cm in length and 10 cm in diameter). Tap water was added to each tube with 5 cm standing water over the soil surface. The tubes were wrapped with aluminum foil, had free contact to air, and were undisturbed at room temperature (22 °C) for 8 months. The soil clearly became stratified during the prolonged flooded condition with an approximate 1 cm yellow surface layer, a 1 cm black layer beneath the surface layer, and underlying dark layers to the bottom.

Soil redox potential of each layer of the stratified soil profile was measured in duplicate using a digital Eh meter (Cole-Parmer Instrument Co. Illinois) with a calomel

reference electrode. Two working platinum (Pt) electrodes were inserted into the soil core from the surface, and were kept 1 day for each layer measurement.

Experimental Design and Measurements

All technical operations with the stratified soil core under different treatments were carried out in a glove bag inflated with pure N_2 to ensure an anaerobic environment. The stratified soil core was divided into 6 layers. The two surface layers were 1 cm each (A and B), and the remaining soil was evenly divided into 4 layers (C, D, E, and F). Approximately same amount of soil from each layer was transferred into a 250 ml beaker, and 30 ml distilled water was added. The soil was homogenized by stirring the soil for 5 minutes to make a soil slurry. Each soil slurry was evenly distributed by syringe to 10 bottles (60 ml in volume) for incubation to determine CH_4 and N_2O production from the soil under different treatments. Each bottled was capped with a rubber stopper through which a gas sample can be taken using a syringe.

Five different treatments in duplicate (Table 6.1) were established in order to determine the CH_4 and N_2O production potentials in the soil. These are: control, carbon source addition, C_2H_2 addition, KNO_3 addition combined with C_2H_2, and external N_2O addition. Dextrose was added through the rubber stopper to provide a carbon source for CH_4 and CO_2 production, which would also function as an electron donor for denitrification. Nitrate was added to provide additional substrate for N_2O production in denitrification. All bottles were flushed with pure N_2 after dextrose and KNO_3 additions. Then 3 ml of acetylene was injected by to the corresponding bottles to inhibit the reduction of N_2O to N_2. Exogenous N_2O was introduced into the bottles to determine the

Table 6.1 Experimental treatment

Treatment	Description
Control	No addition
Dextrose	1 ml of 1 % (w/w) Dextrose solution
C_2H_2	3 ml of 99.9 % acetylene
Nit.+C_2H_2	1 ml of 7.12mM KNO_3
N_2O	1 ml of 3.86 % (v/v) N_2O

N_2O reduction activity at each soil layer. Nitrous oxide, methane, and CO_2 concentration in the headspace of the bottles were analyzed with a Tremetrics 9001 gas chromatograph.

Calculation, Regression and Statistical Analysis

The measurement was conducted in three days with CH_4, CO_2, and N_2O concentration determined once a day. Nitrous oxide, CH_4 and CO_2 production rates were determined by the linear regressions of these three measurements. After the gas measurements, all the bottles were uncovered and put in the oven at 120 °C for 3 days to determined the water content and the soil dry weight. Then headspace of the bottles was calculated individually. All gas production rates in the results were expressed in dry weight of soil, and the amount of N_2O dissolved in the water phase of the slurry was considered in the calculation by taking Bunsen coefficient as 0.556 at 25 °C. Microsoft Excel 7.0 software for Windows 95 was used for the calculation, regression of coefficients between different variations and statistical analysis.

RESULTS

Redox Potentials in The Stratified Soil Profile

The results indicated that the soil redox potentials decreased from the surface to the bottom in the stratified soil profile. Rapid redox potential change took place in the two surface layers. The decrease of redox potential in the remaining four layers was comparatively slight (Figure 6.1). The surface layer was actually in aerobic condition, since the redox potential was up to +350 mV. The bottom layers were so reducing that the redox potentials were below -200 mV.

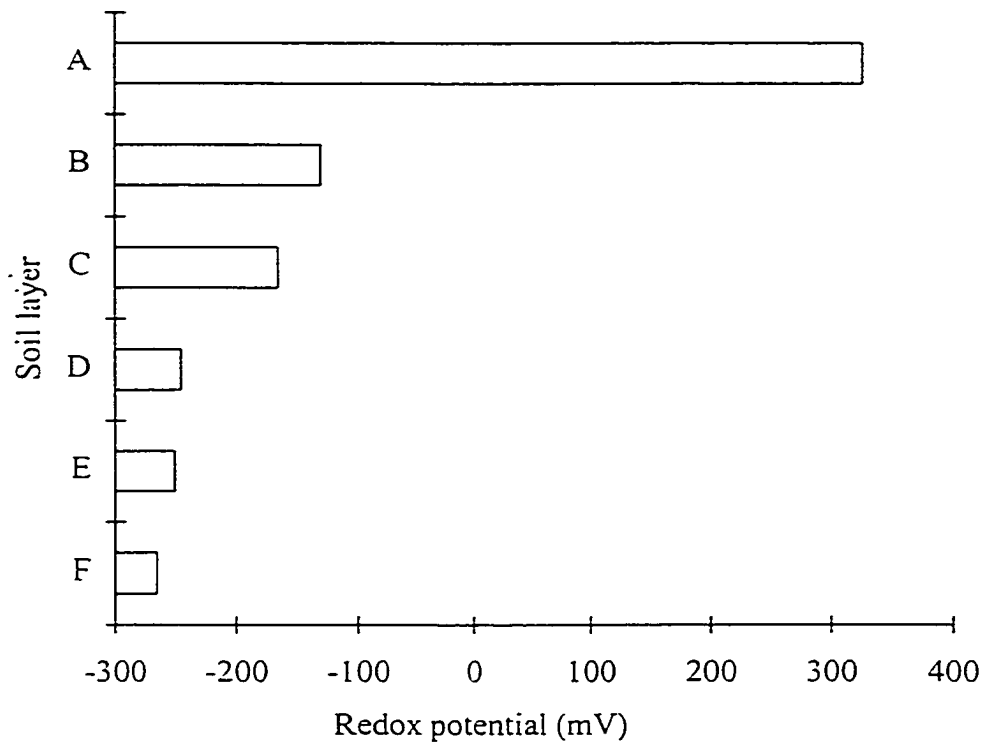


Figure 6.1 Redox potential in the stratified soil profile

Methane Production

The same trends were found in all treatments where CH_4 production rate increased from the surface to the bottom layer of the soil. The rapid change in CH_4 production rate was happening in the middle two layers of the soil where redox potential dropped below -100 mV (Figure 6.2). Methane production was not significantly stimulated by additional carbon source, indicating that CH_4 production was not limited by carbon source.

Nitrous Oxide Production and Reduction

Nitrous oxide production was found in all soil layers (Figure 6.3). With no addition, N_2O emissions in the surface two layers were lower. The remaining layers of the soil showed similar N_2O emission rates. It is important to understand that N_2O emission is the balance of N_2O production and reduction. When N_2O reduction was inhibited by C_2H_2 addition, all soil layers actually showed similar N_2O production, indicating N_2O was actually produced at the same rate in the whole soil profile. The results also showed that the denitrification reaction was limited by nitrate availability in the tested soil, because nitrate addition significantly stimulated N_2O production in each layer of the soil. Greater stimulation by nitrate addition was found in the two surface layers of the soil. The middle layer (layer C) showed only slight stimulation. Organic matter addition did not change the pattern and rates of N_2O emission in the soil profile. Nitrous oxide reduction could occur in all soil layers. Higher N_2O reduction activity was found in the surface layer, which was likely due to the larger N_2O reducer population because the higher redox potential level in this layer was not favorable for N_2O reduction.

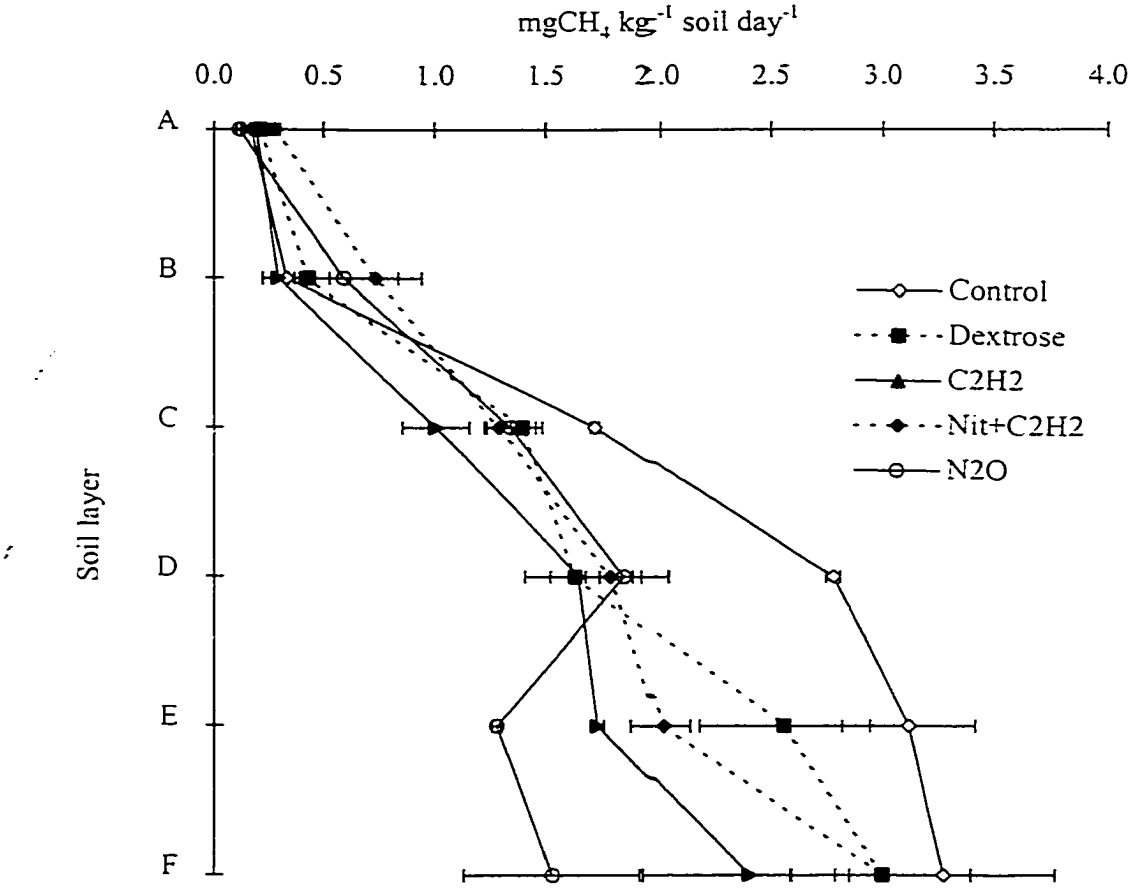


Figure 6.2 Methane production in different layers of the soil

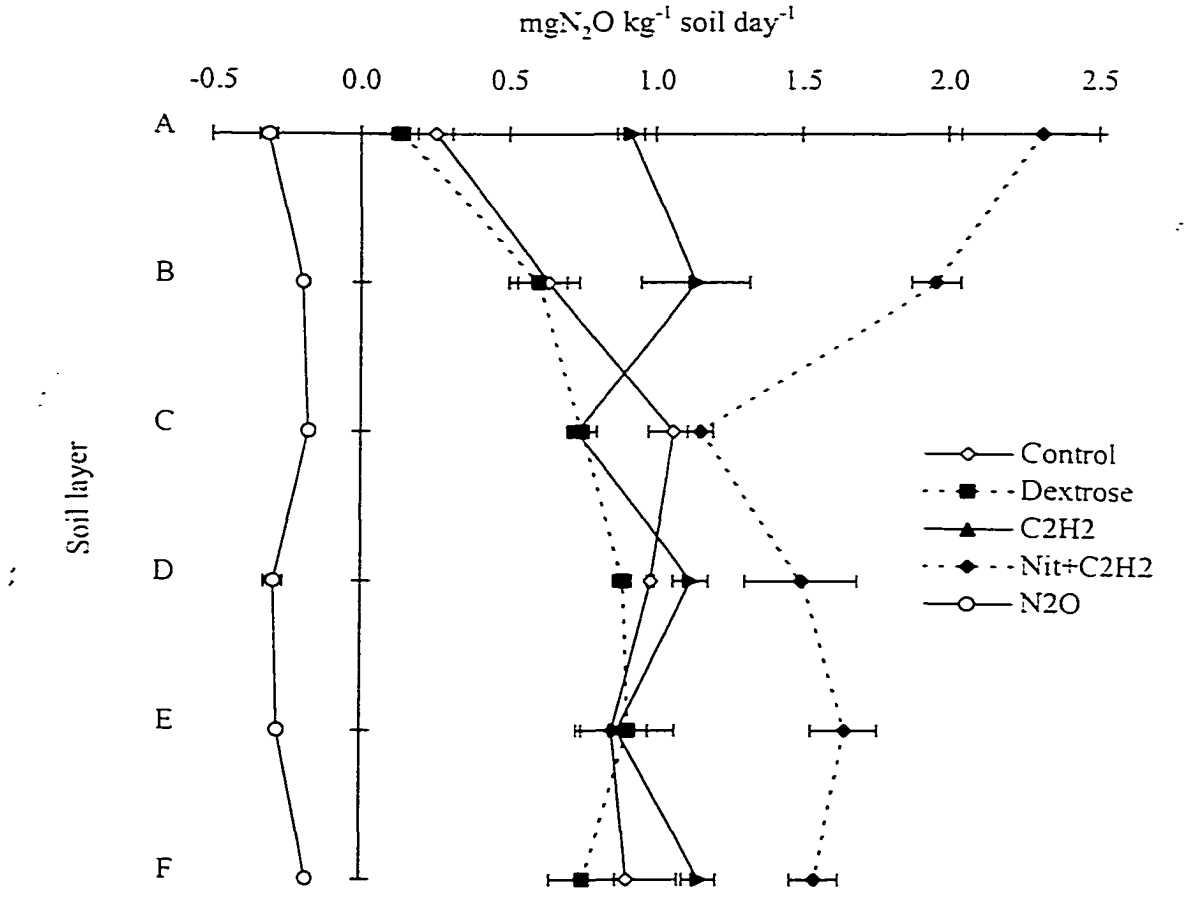


Figure 6.3 Production and reduction of N_2O in different layers of the soil

DISCUSSION

The production and emission of CH_4 and N_2O from a soil are regulated by various physical, chemical and biological factors, among them soil redox potential, availability of substrate (nitrate and carbon source) and microbial community and population are the most important. In an undisturbed soil core, when O_2 diffusion to the soil is limited by a standing water layer on the soil surface, the soil core tends to be stratified regards to redox potential. The different color at each soil layer indicated different oxidation-reduction reactions occurring at different soil redox potential conditions. When plants are not present in the soil, there is only one redox potential profile from aerobic (oxidized) to anaerobic (reduced) conditions.

Methane Production

The amount of CH_4 emission represented the amount of CH_4 production in this study, because CH_4 oxidation took place mainly in aerobic conditions. Redox potential has a critical control on CH_4 production that is a strictly anaerobic process. The critical redox potential for CH_4 production in this rice soil has been previously determined to be about -160 mV in a microcosm study (Wang et al., 1992). However, CH_4 emissions actually occurred in all soil layers, especially below the C layer where soil redox potential was less than -100 mV. The small amount of CH_4 emission detected from the surface layer was possibly due to the early initiation of CH_4 production, the decrease of soil redox potential during the anaerobic incubation, or due to the entrapment of CH_4 diffused from deeper layers of the soil before the stratified soil was transferred into the incubation bottles. Same trends were found in all treatments — CH_4 production rate increased from

the surface to the bottom layer, indicating that CH_4 production was strongly related to soil redox potential. Addition of a carbon source did not enhance CH_4 production, indicating organic matter was not a limiting factor for CH_4 production in this study. However carbon source stimulated CO_2 production in the bottom layer. It was likely related to the highest CH_4 production rate in this layer, which decreased the organic matter content level. When comparing the whole soil profile, three was no significance difference in CO_2 production rate between different treatments (Table 6.2). Acetylene and N_2O additions inhibited CH_4 production significantly. The implication of N_2O on soil anaerobic oxidation and reduction reactions was discussed in detail in Chapter V. Thus, the inhibition of CH_4 production by N_2O was probably due to two mechanisms: (1) increase of soil redox potential by the presence of N_2O; (2) decrease of methanogen population by exposure to N_2O that has a cyto-toxic effect. The inhibition effect of acetylene could be indirectly from the buildup of N_2O concentration by blocking N_2O reduction to N_2. It is also important to be aware of the presence of NO when denitrification reaction is happening, but it is beyond the scope of this dissertation study.

Nitrous Oxide Production and Reduction

Denitrification was the only source of N_2O production in the soil column, because the experiment was conducted in anaerobic environment. Denitrification also represents the only biological mechanism of N_2O consumption. Both N_2O production and reduction are influenced by soil redox potential. Higher N_2O production and reduction rates are reported at lower soil redox status (Smith et al., 1983; Kralova et al., 1992). More information on the relationship between soil redox potential and N_2O production and reduction was presented in Chapter III. In this study, N_2O was produced at similar rate

Table 6.2 Average production rate of N_2O, CH_4 and CO_2 in the soil core

Treatment	Average production rate (mg kg^{-1} soil day^{-1})		
	N_2O	CH_4	CO_2
Control	0.78	1.90	14.69
Dextrose	0.67	1.54	16.53
C_2H_2	0.99	1.22 *	13.80
Nit.+C_2H_2	1.68*	1.52	12.46
N_2O	-0.24 **	1.12 *	12.68

* (95 % level) and ** (99 % level) indicate the labeled values are significantly different than control

(in C_2H_2 inhibition treatment) in the whole soil profile even though soil redox potential continuously decreased from the surface to the bottom. Substrate control was another important factor affecting N_2O production in denitrification. Highest N_2O production was found in the surface layer when additional nitrate was provided. It was likely due to larger denitrifier population existing in the surface layer where on the other hand nitrate was limited for denitrification to function. There was a probably decrease tendency in denitrifier population from the surface to the bottom of the soil, because denitrification potential decreased from the surface to the middle layer while redox potential was becoming more favorable for denitrification. Denitrification rates were enhanced by the favorable lower soil redox potential in the bottom layers even where denitrifier population was probably smaller. Due to the combined effect of denitrifier population and soil redox potential, such a particular pattern of denitrification potential in the soil profile was formed when denitrification was not limited by nitrate. The higher denitrification potentials in the surface and bottom layers were due to the effect of larger denitrifier population and lower soil redox potential, respectively. Lowest activity was found in the middle layer (C layer) where the population of denitrifier was lower than the surface layers, while soil redox potential was higher than the bottom layers.

Larger N_2O reducer populations possibly also existed in the surface layers. Although the aerobic environment in the surface layer was not favorable for N_2O reduction, lowest N_2O emission was found in this layer when N_2O reduction was not inhibited by C_2H_2. Nitrous oxide reduction activity was so low between C to F layers that there was no significant difference in N_2O emission between treatments with and without C_2H_2 inhibition. Organic matter addition could enhance both N_2O production and N_2O

reduction, because both of these two processes needed to accept electrons to complete the reactions. In this study, dextrose addition decreased the N_2O emission rate by 14 % (Table 6.2). Since this is the finale result of both N_2O production and reduction, it suggested that extra organic matter could favor N_2O reduction over N_2O production in this case.

CHAPTER VII MITIGATION OF METHANE AND NITROUS OXIDE EMISSIONS FROM AN IRRIGATED RICE FIELD BY CONTROLLING SOIL REDOX STATUS

INTRODUCTION

Methane and nitrous oxide are two important greenhouse gases of mostly biotic origins (Wahlen et al., 1989; Duxbury et al., 1993). Flooded rice fields are one of the most important anthropogenic sources of atmospheric CH_4 and a potential important source of N_2O. Methanogenesis (contribution to CH_4 production), nitrification and denitrification (contribution to N_2O production) are soil microbiological processes affected by many physical and chemical factors, such as soil pH, temperature and soil water content, etc. Soil oxidants (O_2, NO_3^-, Mn^{4-}, Fe^{3-}, SO_4^{2-} and CO_2) function as electron acceptors for organic matter degradation contribute significantly to these processes. The reduction of various oxidants in homogeneous soil suspension occurs sequentially at each corresponding soil redox potential level (Ponnamporuma, 1972; Patrick and DeLaune, 1977). Both CH_4 and N_2O production are related to soil redox potential and microbiological processes. Methane production rate is generally high in flooded soils (usually reflected by low soil redox potential) with high organic matter content. These soils can also produce N_2O when they are not constantly flooded because of the availability of mineral-N and the temporary oxidized condition that enables nitrification to occur (Byrnes, 1993). Reduced duration of flooding field increases N_2O production, whereas prolonged flooding makes soils maintain anaerobic conditions and consequently enhance CH_4 production (Neue, 1993). The factors affecting CH_4 and N_2O emissions are complicated and internally related. New information on such relationship is

needed as agricultural practices such as irrigation and organic manure management, and changing fertilization method might be applied to mitigate the emission of these two greenhouse gases. The trade-off relationship of CH_4 and N_2O emissions from flooded rice fields deserves special attention when modified management practices are proposed, and its ecological consequence needs careful evaluation.

The biggest problem in mitigation of greenhouse gas emissions from rice fields arises from the globally increasing food demand. An increase of rice production by 60 % is the most appropriate way to sustain the estimated increase of the human population during the next three decades (Cassman et al., 1998). Irrigated rice has the highest CH_4 source intensity because of the flooded condition and the area planted. Highest CH_4 fluxes are observed in fields receiving organic amendments. Lowest CH_4 fluxes are recorded in fields with low residue recycling, multiple aeration periods, poor soils and low fertilization with resulting poor rice growth and low yields. It is a great challenge to keep high rice production yields while minimizing CH_4 emissions from rice fields. Four principles have been proposed to follow in recommending a practice to reduce CH_4 emissions from flooded rice: (1) yield should not be decreased, and probably increased by a mitigation practice; (2) there should be some additional benefit to the farmer, such as better water utilization or reduction of labor; (3) the rice varieties used should be desired by local consumers; and, (4) the mitigation practice should not increase emissions of other greenhouse gases, particularly N_2O (Mosier et al., 1998). In this field study, the aim is to propose a field management practice following the above principles to minimize both CH_4 and N_2O production and emission in the rice field by controlling irrigation and

organic manure application. Rice yields under different management were evaluated after harvest, but rice varieties were not considered in the study.

MATERIALS AND METHODS

Experimental Site and Treatments

The field experiment was conducted at Shenyang Experimental Station of Ecology, Chinese Academy of Sciences (41° 32' N, 122° 23' E). The soil type is classified as meadow brown. Major physical-chemical characteristics of the soil are shown in Appendix III. Just before the field experiment, the soil organic matter contents were 21.2 g kg^{-1} in the plots receiving organic manure before transplanting, and 15.1 gkg^{-1} in the plots that had not received organic manure for about 10 years.

A single rice species, Liao Kai 79, is cultivated in this region. According to local management, rice was flooded with a standing water layer of 5 to 10 cm during the irrigated period. Organic manure (cattle waste) was applied at 31,000 kg ha^{-1} before flooding the fields. Rice seedlings were transplanted (late May) the second day after flooding, and was fertilized with $(NH_4)_2HPO_4$ at 292 kg ha^{-1} within 3 days after transplanting. Urea was broadcast at tillering (156 kg ha^{-1}, late June) and heading (73 kg ha^{-1}, late August) stages, respectively. The rice field was drained in late September in order to harvest in early October. Two different organic manure application were applied to each of two irrigation practices to the experimental plots (4 × 6 m each). Treatments with application of organic manure included one with normal irrigation to keep the fields flooded as a conventional management practice, the other was to irrigation-controlled plots where irrigation was just enough to keep the soil surface wet as necessary. The

above two irrigation practices were also applied to the field plots that did not receive organic manure. The same inorganic fertilization applications were applied to all four treatments. Each treatment has duplicate plots.

Measurement, Analysis and Calculation

Methane and nitrous oxide emissions in the rice fields under different treatments were measured at least once a week using the static chamber technique (Figure 7.1). The chamber is 1 m high with base area of 0.8 x 0.8 m. During the measurement, the upper chamber assembly was mounted, and was sealed for 40 min with water in the channel of the base. Gas samples were collected in duplicate using a 30 ml syringe at 0, 20, and 40 min upon chamber enclosure. The field measurements were conducted from early July until late September, covering 84 days of the rice growing season.

Redox potentials in the soil profile were measured by a platinum (Pt) electrode cables with a calomel reference electrode. The Pt electrode cable consisted of six single Pt electrodes that was located at different intervals to measure soil redox potentials at 1, 2, 4, 8, 14, and 22 cm from surface to bottom of the soil. Each plot had 4 electrode cables permanently installed during the studied period.

Nitrous oxide, methane, and carbon dioxide concentrations at different depths of the soil were studied using pore-water equilibrator. The pore-water equilibrator was constructed of acrylic blocks with 12 sampling cells (cell volume=15 ml). Each cell is 1 cm wide and was distributed 1 cm apart in the block. The cells was filled with deionized water, covered with a thin permeable membrane (Poretics, 0.2 micron), driven into the soil and left for at least two weeks. This allowed time for the device to equilibrate with dissolved solutes and gases in the soil. Upon removing the pore-water equilibrator from

the field, about 6 ml of water in the sampling cells was immediately taken using a syringe and transferred into an evacuated vial (10 ml Vacutainer, Becton Dickinson, New Jersey, U.S.A.). The remaining volume of the vials was filled with pure nitrogen of the normal atmospheric pressure. Each plot had one pore-water equilibrator, and water samples were taken in duplicate. This measurement was conducted three times at different growing season. Nitrous oxide, methane, and carbon dioxide concentrations in the vials were analyzed using gas chromatography after shaking for 4 hrs to equilibrate the gas in the headspace with those dissolved in the water phase. Nitrate and ammonium dissolved in the water were also analyzed.

Methane was analyzed with a HP-5890 GC with FID detector. Nitrous oxide and CO_2 were analyzed by a Shimadzu GC-14A with ECD detector. Standard gases of CH_4, N_2O, and CO_2 were provided by National Research Institute of Standard Material, China. Soil redox potentials were measured by a portable redox meter (Digi-Sense 5938-00, Cole-Parmer Instrument Co.) with a calomel reference electrode. Methane and nitrous oxide emissions in the rice field are calculated by linear regression of their concentrations against time. Nitrate and ammonium were measured by distillation in presence of MgO and Devarda's alloy.

Results and Discussion

Methane and N_2O emissions in this rice field have been monitored for a couple of years. Previous studies mainly focused on the seasonal and yearly variations of the CH_4 and N_2O emissions, and the trade-off relationship between these two gases (Chen et al., 1995 and 1997). The CH_4 emission is the net result of opposing bacterial processes, production in anaerobic micro-environments, and consumption (oxidation) in aerobic

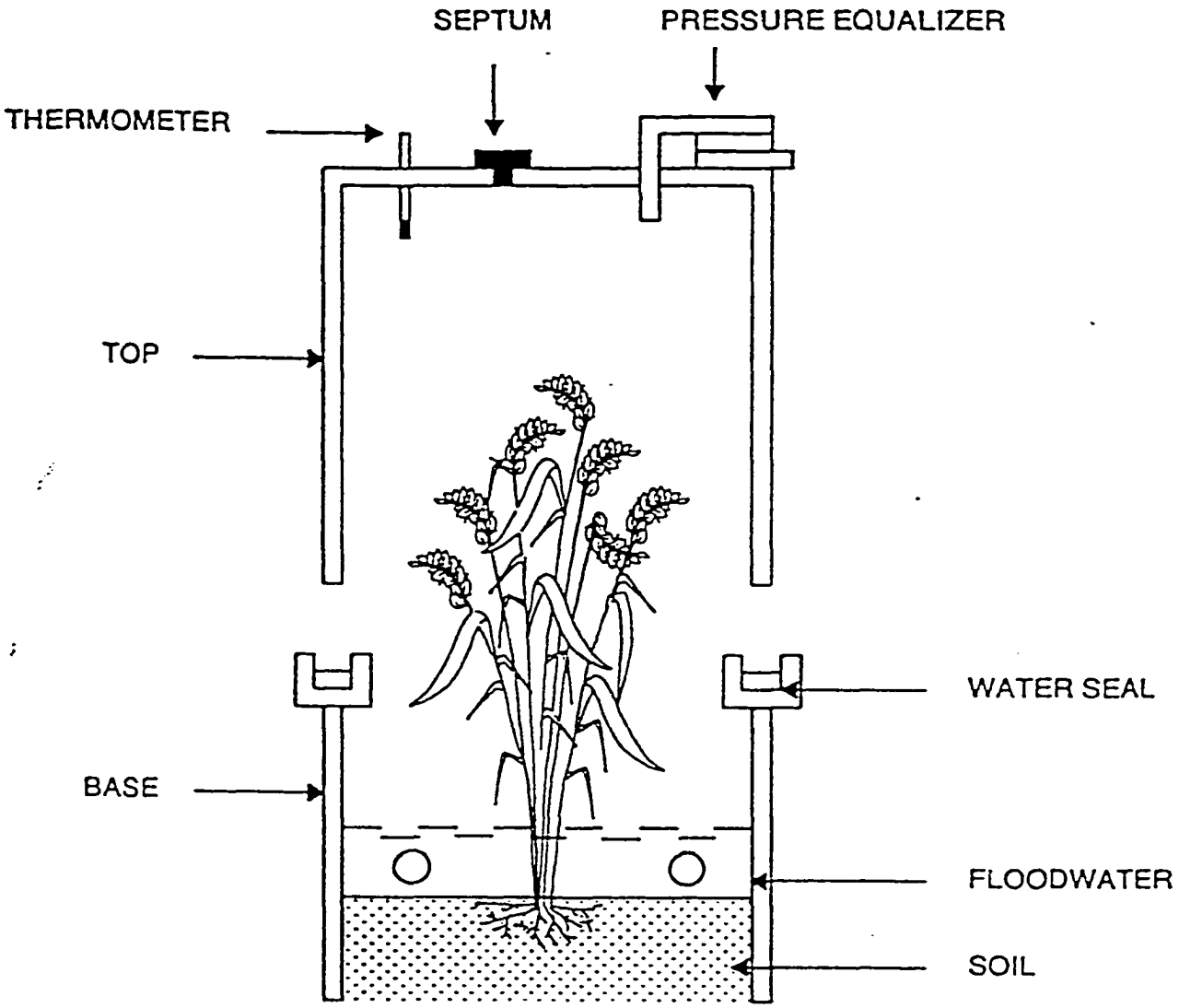


Figure 7.1 Closed chamber for CH_4 and N_2O measurement in fields

micro-environments. Total CH_4 fluxes from rice fields also depend on its transport from soil to the atmosphere.

Methane and Nitrous Oxide emissions

The seasonal patterns of CH_4 and N_2O emissions in this study agree quite well with the previous measurements, but with some variations in the flux rate (Figure 7.2 and 7.3). The seasonal variations of CH_4 emission from rice fields are mainly controlled by two factors. One is CH_4 production from degradation of original soil organic matter and new released organic matter from rice roots. Organic substrate released by rice plants will stimulate stronger methanogenesis activity, which will consequently increase the concentration gradient and accelerate CH_4 emission. Isotopic analysis of dissolved CH_4 in peat-pore water and emitted CH_4 suggests that a large fraction of the organic material that supports methanogenesis is indeed derived from recently fixed carbon (Chanton et al., 1995). The other factor is the mechanisms by which CH_4 is transported from soils to the atmosphere. Methane produced in waterlogged, anoxic environments can escape through the soils or sediments into the atmosphere either by diffusion, bubble ebullition or transport through vascular plants. To a large extent, the type of transport pathway controls the total CH_4 emission rate. It has been shown that the tillers are responsible for more than 90 % of the total CH_4 flux in rice fields (Hollzapfel-Pschorn et al., 1986; Schutz et al., 1991). The entrance of CH_4 into the aerenchyma of the plant roots is facilitated by a diffusion gradient between the sediment and the atmosphere in the opposite direction to that of O_2 (Schutz et al., 1991). Both molecular diffusion and bulk flow are responsible for the aeration of the submerged organs, leading to stimulated transport of CH_4 to the atmosphere. The effect of vascular plant transport is a double-

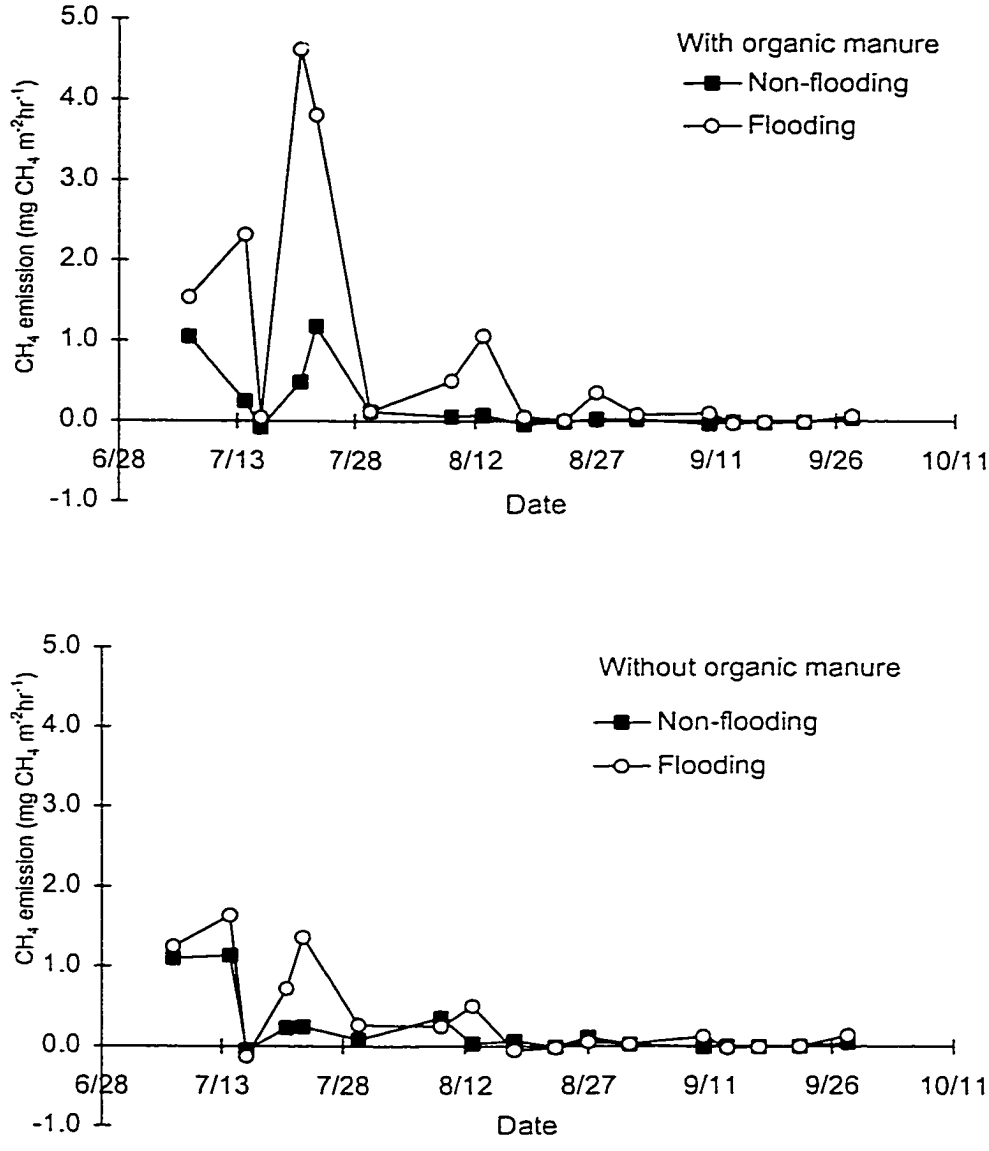


Figure 7.2 Effects of organic manure and irrigation on CH_4 emissions in rice field

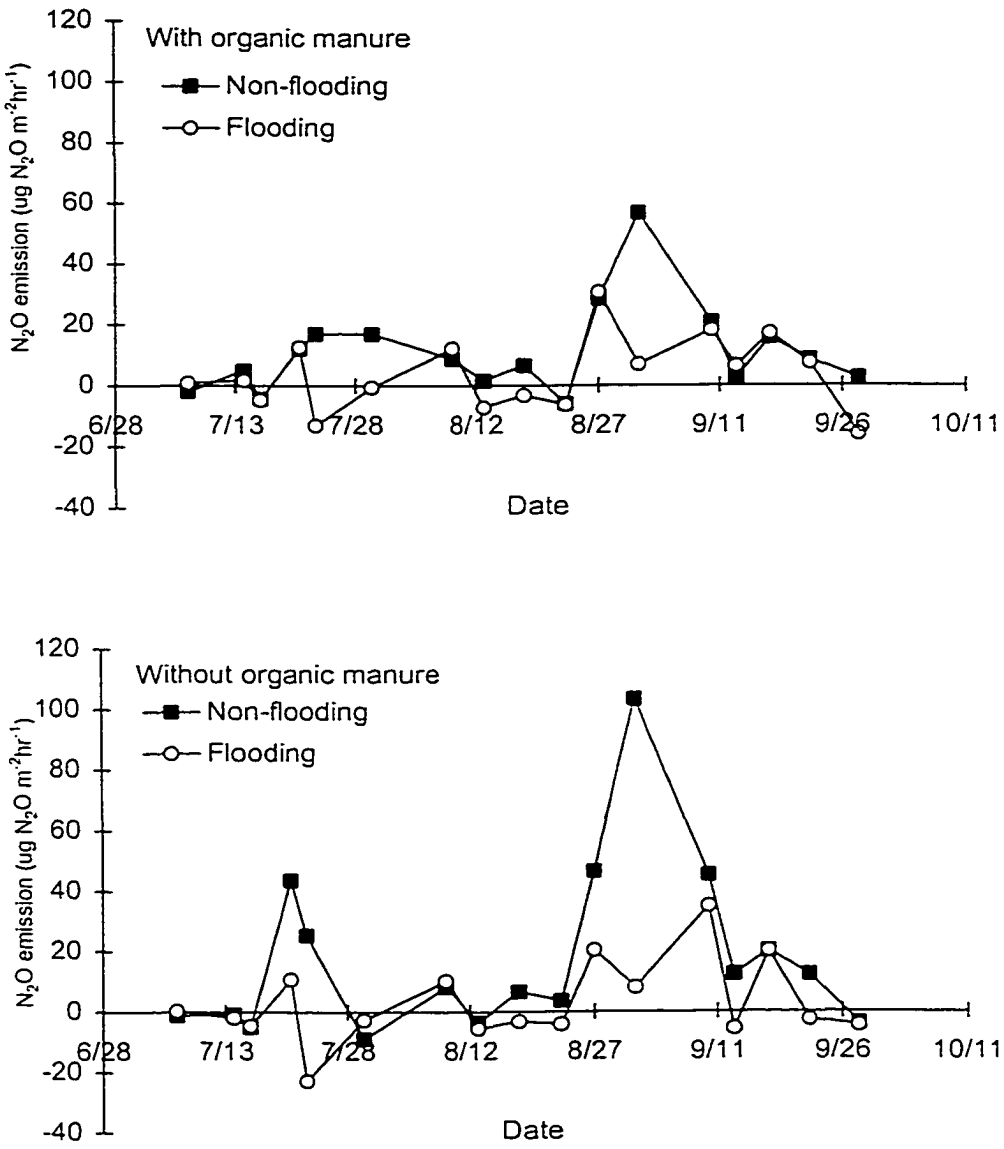


Figure 7.3 Effects of organic manure and irrigation on N_2O emissions in rice field

edged sword with respect to the effects on net CH_4 emission. On the one hand, plant roots decrease CH_4 production through O_2 transport to the rhizosphere, because the strictly anaerobic methanogenic bacteria are thereby inhibited. In addition, aerobic CH_4 oxidation might also be stimulated, since the activity of methanotrophic bacteria is constrained by O_2 transport from the roots to the otherwise anoxic rhizosphere (Watson et al., 1997; Chanton and Dacey, 1991). On the other hand, however, there are indications from simulation models (Watson et al., 1997) that the net effect of the presence of plant roots on the ultimate CH_4 emission is that the CH_4 flux to the atmosphere is increased compared with a situation where no plant roots are present.

This study focused on how to minimize CH_4 emissions without enhancing significant N_2O emission in a rice field by controlling soil redox potentials. Ideally, an approach to control greenhouse gas emissions in rice fields should not bring any adverse effects on rice yield. Different management of irrigation and organic manure application in this study showed a significant effect on CH_4 emissions in the fields. Highest CH_4 flux was found in the treatment of flooding the field and organic manure amendments. Application of organic manure increased CH_4 emissions by about 130 % when the rice field was flooded, and by about 70 % when the fields were irrigated to wet condition but not flooded. Irrigation control showed more effective reduction in CH_4 emissions, especially when CH_4 emission was higher in the plots with organic manure (Table 7.1). This was due to the relative easy availability of O_2 in non-flooded condition, which mainly had two effects: when O_2 is available, (1) most of the easily degraded organic matter are likely converted to CO_2 by aerobic microbial processes instead of being converted to CH_4 by anaerobic microbial processes; (2) CH_4 produced in anaerobic

Table 7.1 Average fluxes of CH_4 and N_2O from rice fields in the growing season (n=2)

	With organic manure		Without organic manure	
	Non-flooded	Flooded	Non-flooded	Flooded
CH_4 (mg m^{-2} hr^{-1})	0.22	1.05	0.13	0.45
N_2O (μg m^{-2} hr^{-1})	5.86	1.93	9.38	1.50

micro-sites of the soil are more likely to be oxidized to CO_2 in non-flooded field plots before it evolves from the soils to the atmosphere.

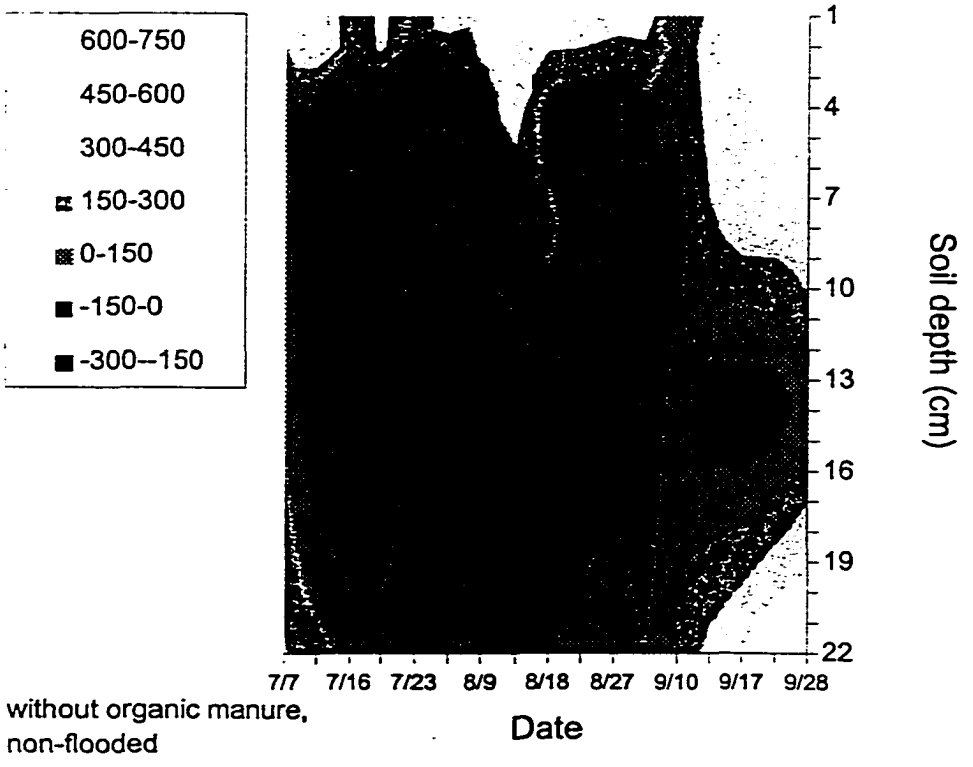
Denitrification is most likely the major source of N_2O emission in this study, because the soil did not experience aerobic conditions except during a short period of complete drainage before harvest. Both soil denitrification rate and N_2O/N_2 production ratio must be known to evaluate the N_2O emissions through denitrification. Soil structure, water content, microbial populations, and available C are important factors determining the proportions of these two gases which affect the balance between diffusive escape of N_2O and its further reduction to N_2. Nitrous oxide emission was generally low in the studied rice fields. Higher N_2O emission was stimulated when urea was added, possibly due to the coupling of nitrification and denitrification (Figure 7.2 and 7.3). Nitrous oxide emissions in the non-flooded condition were several folds higher than that in flooded condition. However, organic manure addition could help to prevent too much increase of N_2O emission by irrigation control (Table 7.1).

The possible approach proposed in this study to minimize CH_4 and N_2O emissions is to keep rice soil non-flooded but wet during the rice growing season. Organic manure is strongly recommended to be applied before transplanting of rice seedling. This management will significantly reduce the labor and irrigation cost, which makes it economically feasible. The results also suggested that it could be possible to reduce the short-term higher N_2O emission due to inorganic fertilization by temporally flooding the fields, but it has not been investigated if such practice will result in higher CH_4 emission in the fields at the same time. Mitigation of greenhouse gases in rice fields had been generally restricted to intermitted drainage during the rice flooding season. A new

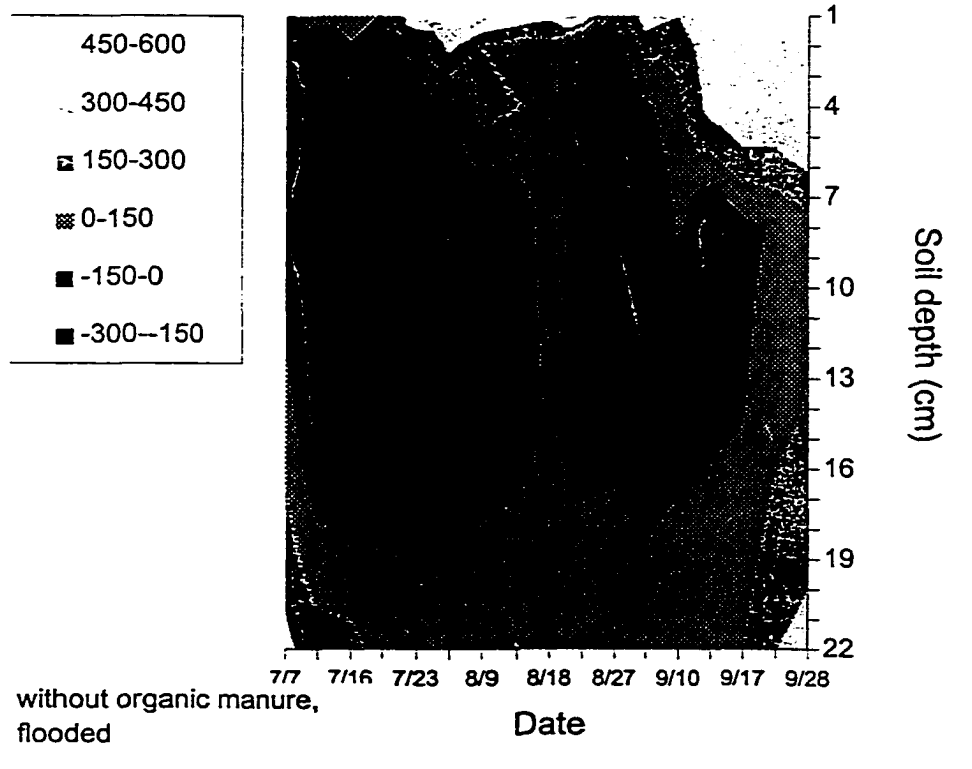
management approach was proposed in this study and proved to be a significant improvement compared to the other mitigation technique. It is not difficult for farmers to control the irrigation to make the fields non-flooded but wet. Flooding the field by irrigation after inorganic fertilization during the rice growing season is widely used for the purpose of improving fertilizer efficiency. Irrigation control combined with fertilization (organic and inorganic) could be a practical approach to minimize both CH_4 and N_2O emissions from the rice fields without influencing rice yield (see following section).

Soil Redox Potential During the Rice Growing Season

The previous laboratory experiment of this dissertation study showed that soil redox potential has a dominant control of CH_4 and N_2O emissions from soil suspensions (see Chapter IV). In this field study, the aim is to find a management option that can control soil redox potential in a desirable range to minimize both CH_4 and N_2O emissions from the rice fields. In an actual rice field, it is impossible to control the soil redox potential within a narrow range as in a soil suspension because of the heterogeneous nature of the soil, different O_2 availability and demand, the presence of rice roots, and the time required for water addition to result in lower redox and water losses to result in higher redox as water levels fluctuate. However, the results showed that this different management practice did have a profound impact on the soil redox status (Figure 7.4 and 7.5). Soil redox potential was generally lower in the flooded plots because O_2 diffusion from atmosphere into the soil profile was limited by the standing water layer. Application of organic manure also resulted in a lower soil redox status by facilitating soil oxidation-reduction reactions going to the reduced sides. During the study period,

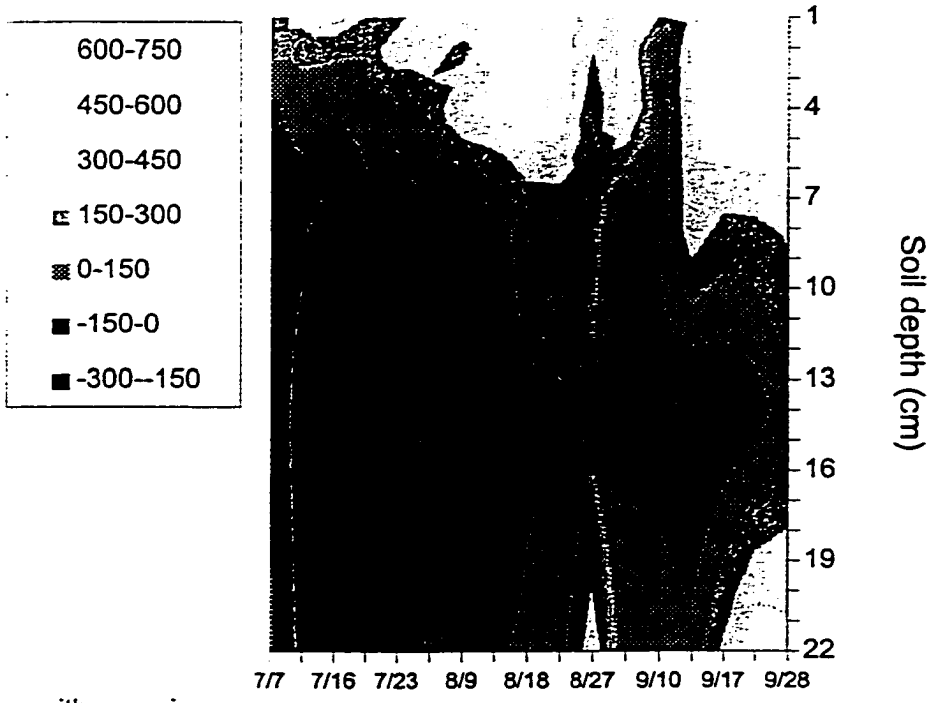


without organic manure, non-flooded

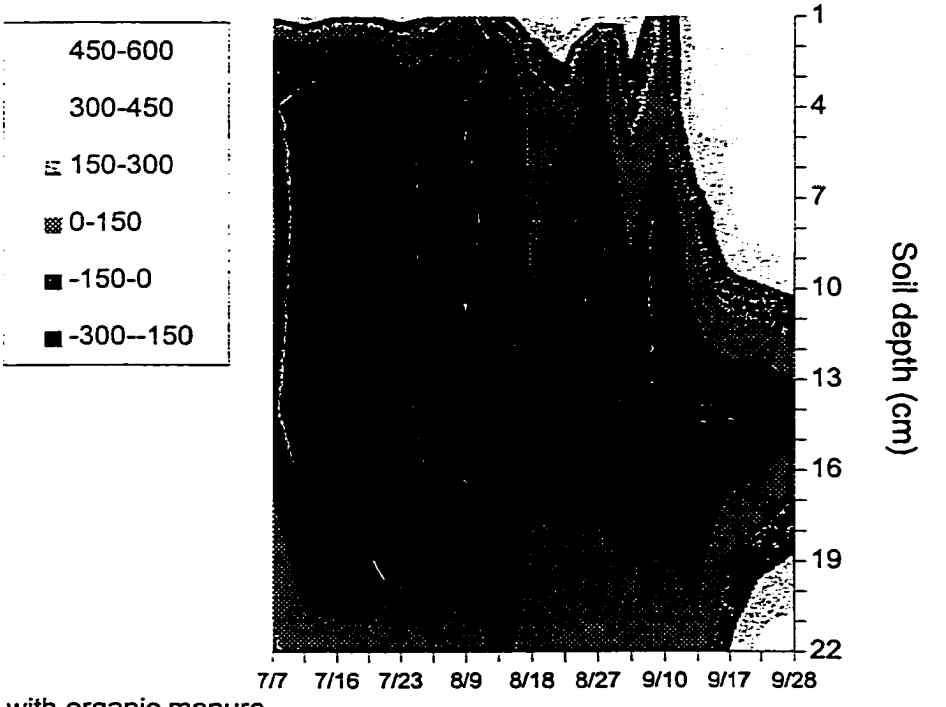


without organic manure, flooded

Figure 7.4 Soil redox potentials (mV) in the rice plots without organic manure application



with organic manure, non-flooded



with organic manure, flooded

Figure 7.5 Soil redox potentials (mV) in the rice plots with organic manure application

strongly reducing conditions indicated by soil redox potential being lower than -150 mV were developed following the degradation of organic matter in different plots. This strongly reducing zone was generally located between 2 to 16 cm from surface to bottom of the soil. This redox pattern was quite different from the flooded soil core where it was always the deeper the soil, the lower the soil redox potential (see Chapter VI). It was likely because of the effect of rice plant root. Oxygen supply from rice plant root was significantly facilitated after the root aerenchyma system developed. Oxygen diffused in all direction from rice rhizosphere, but encountered different O_2 demand. Microbial activities were probably weaker in the soil below root zones, resulting in higher redox potential. Another source of O_2 supply would be irrigation water or possibly ground water where dissolved O_2 might be found, because there is not enough organic matter and microbes to consume the O_2 and other soil oxidants.

The seasonal patterns of the soil redox potential change were similar in differently treated plots. Original soil organic matter and new released organic matter from rice plant roots play an important role in the redox status of the soil profile, which is closely related to the seasonal CH_4 production and emission. The flooding and draining cycle is another factor to control soil redox potential. When soil was flooded in the plots with organic manure, the strongly reducing zones where significant CH_4 were produced were located just 2 to 3 cm below the soil surface. In such situation, CH_4 produced was more ready to emit to the atmosphere by diffusion and ebullition. In non-flooded plots, the strongly reducing zones were located 7 to 8 cm below the soil surface, which provided more chance to oxidize the CH_4 produced in its path from soil to the atmosphere. A

similar pattern was also observed in plots without organic manure, but was not as clear as in the plots with organic manure.

Methane and Nitrous Oxide in Vertical Soil Profile

The measurements to investigate the dissolved gases and solutes in soil pore water at different depths were conducted three times (Figure 7.6, 7.7 and 7.8). Soil redox potentials were found to be consistent with the seasonal measurement, with the lowest soil redox potential generally between 10 to 15 cm below the soil surface. Methane and CO_2 followed the same trend in which their concentrations increased with decreasing soil depth. Major source of CH_4 might be produced in the rice root zone, because of the lower soil redox potential and availability of easily degradable organic matter from root exudates. However, the maximum accumulation of CH_4 was not found in this zone. It might be due to the oxidation of CH_4 in this zone and the easy release of CH_4 through the vascular system of rice root. The vertical movement of CH_4 produced in the rice root zone experienced two different fates. The upward movement of CH_4 would experience more chances of consumption by oxidation with soil oxidants, and the remaining CH_4 emitted from the soil surface into the atmosphere. Thus, CH_4 concentration in the soils decreased from the rice root zone to the soil surface. The downward movement of CH_4 would have less chance of oxidation because of weaker microbial activities in the deeper layer of the soil. In addition, the downward diffusion could be a slow process because of the high density of the deeper soil. Therefore, CH_4 tended to accumulate in the deeper layer of the soil.

Nitrous oxide concentrations in soil pore water did not show such a clear relation with soil depth. In contrast to the CH_4 pattern, N_2O tended to have several accumulation

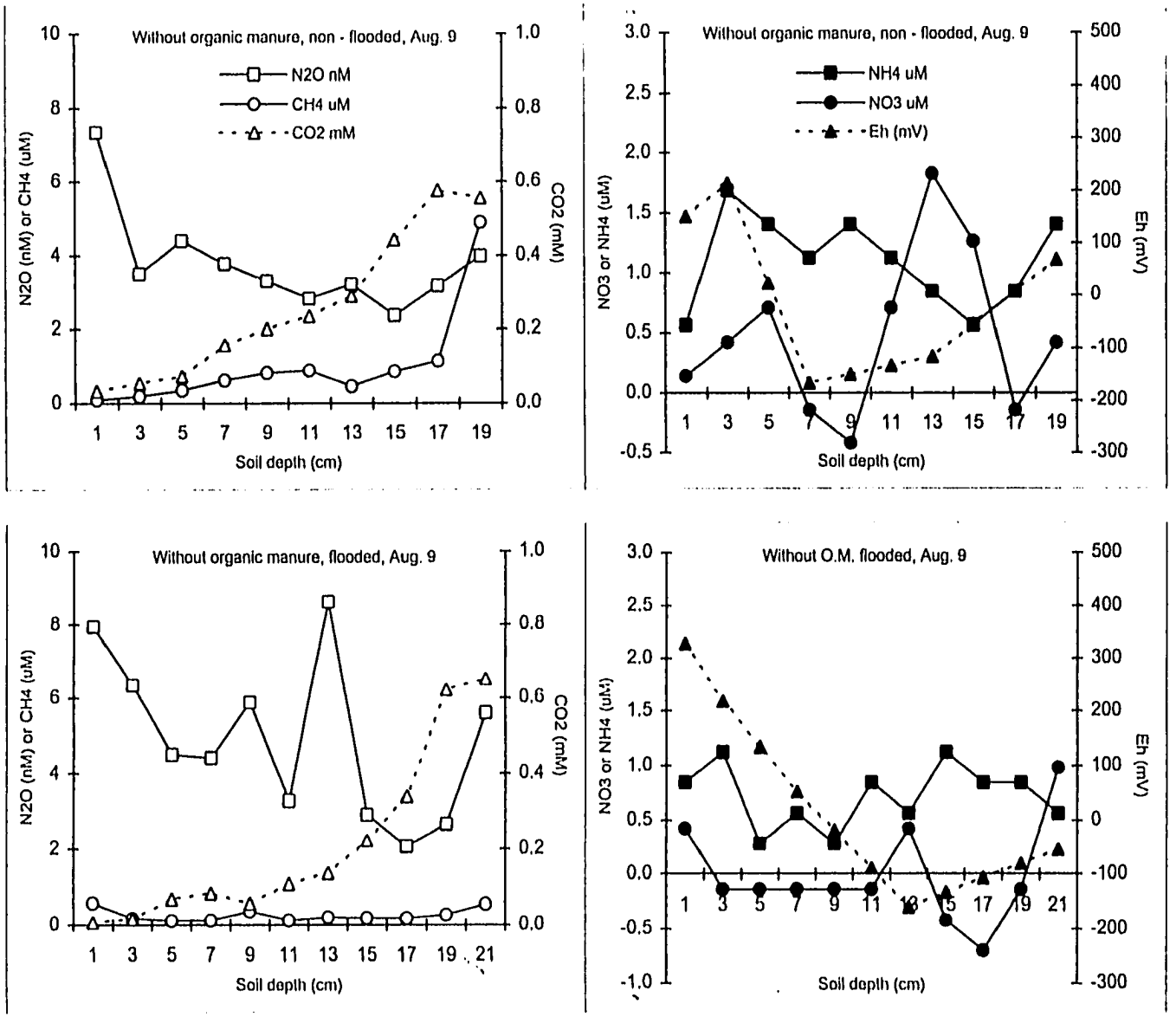


Figure 7.6 Dissolved gases and N solutes in the soil pore water measured on August 9

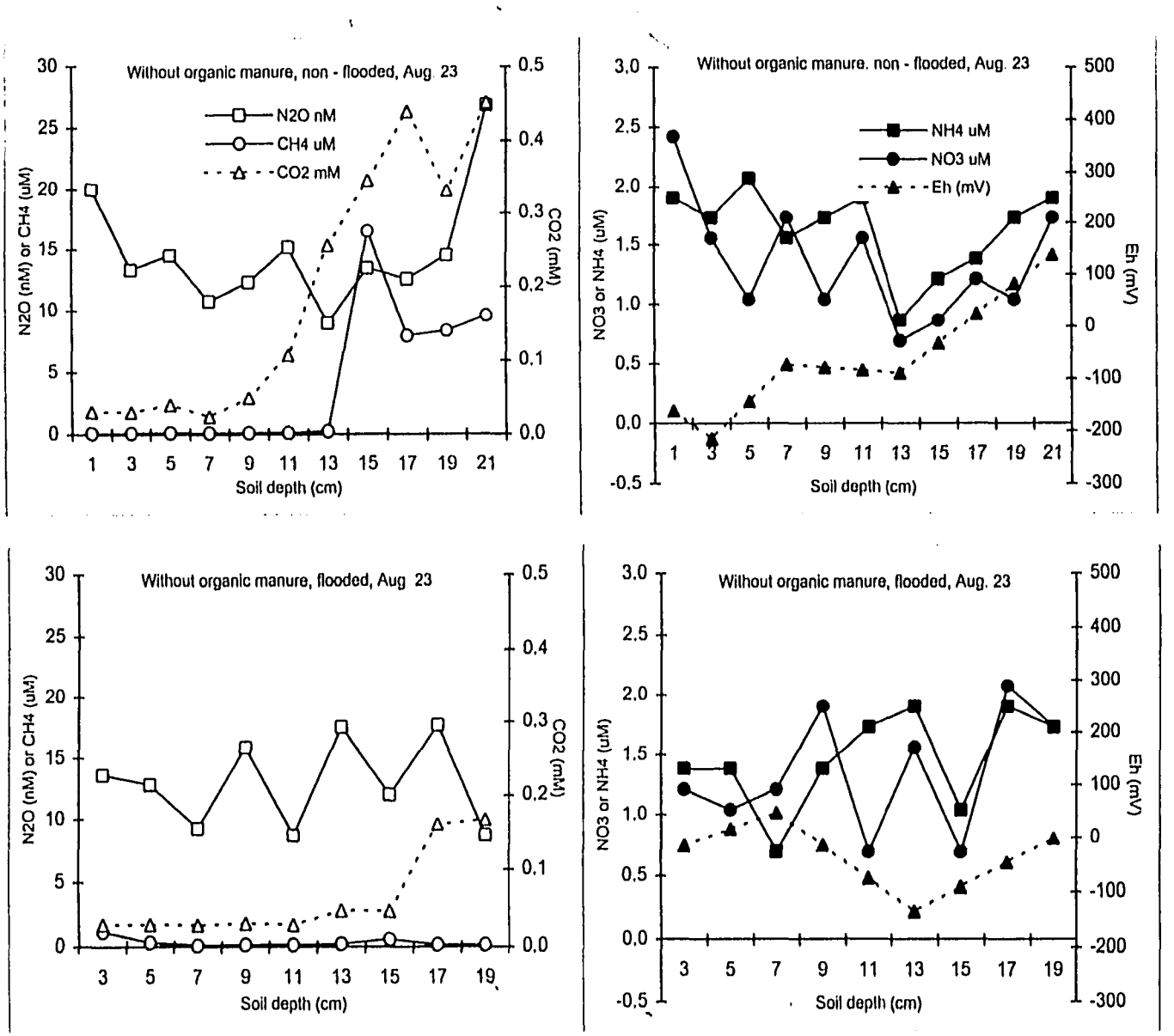


Figure 7.7 Dissolved gases and N solutes in the soil pore water measured on August 23

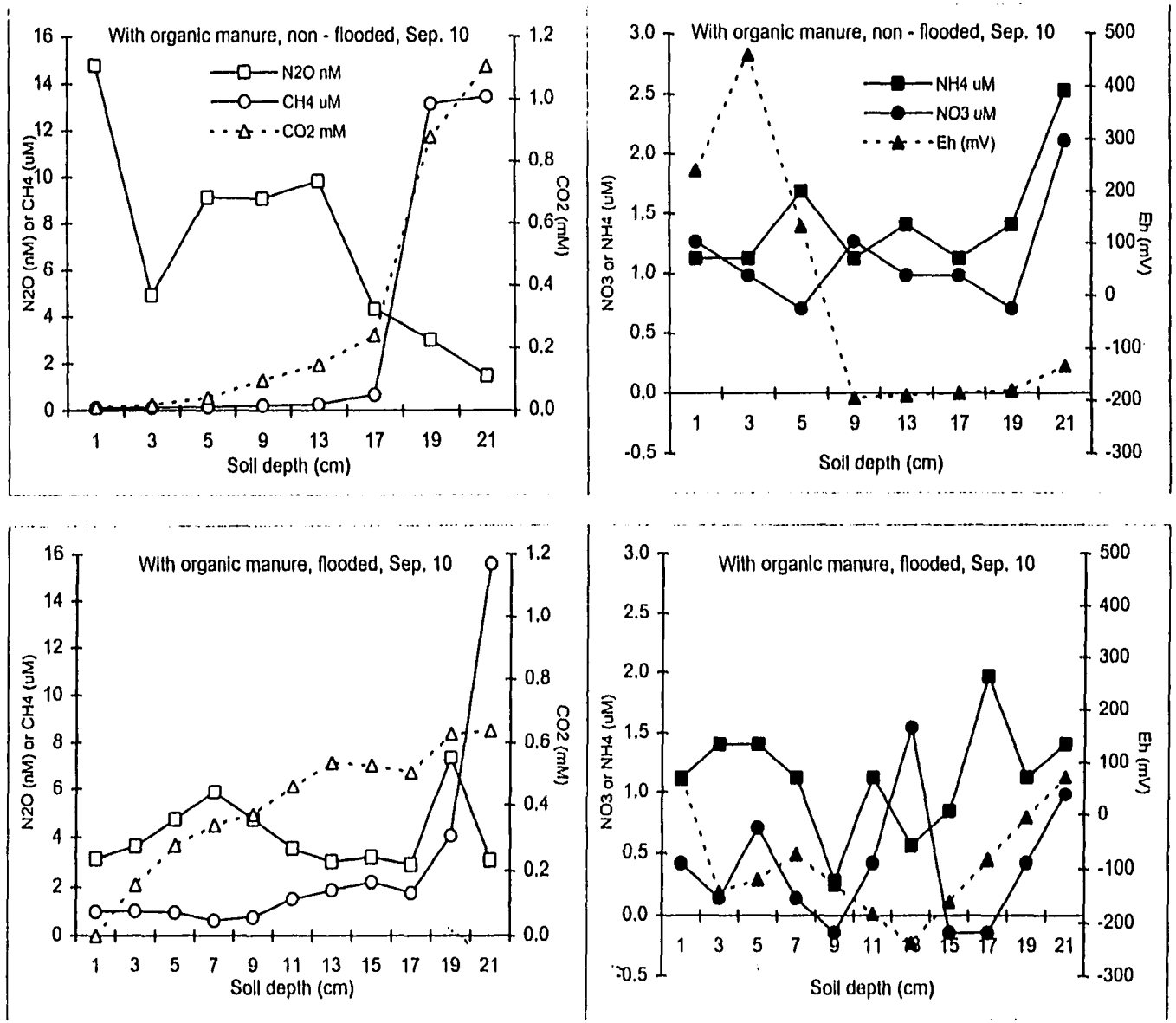


Figure 7.8 Dissolved gases and N solutes in the soil pore water measured on September 10

peaks. Multiple N_2O concentration maxima in the ocean has been reported (Naqvi, 1991; Naqvi et al., 1998). The mechanism is probably the same in which it is due to the combined effect of soil redox status and movement of nitrate in the soil (or water) profile on N_2O production and reduction activities. Soil nitrate and ammonium contents generally followed the same trend in the vertical profile, indicating a close linkage of ammonium and nitrate. The measurement on September 10 was intentionally conducted to study the effect of inorganic fertilization on CH_4 and N_2O productions. The results showed a stronger transformation of ammonium to nitrate by nitrification activity in non-flooded plots where the soils were more oxidizing. The coupling of nitrification and denitrification were probably responsible for the higher concentration of the N_2O in the soil pore water (Figure 7.8), and the higher flux of N_2O to the atmosphere (Figure 7.3). In the non-flooded plots, denitrification process tended to be incomplete due to higher redox potential level and higher nitrate content. Methane showed an inverse relationship with the N_2O production and concentration in the soils. One of the reasons could be the increase of soil redox potential by the presence of temporal large amount of N_2O in the soils (see Chapter V for detail).

Rice Yield

Different agricultural practices have a significant impact on rice yield, which is also an essential part of this study. In this preliminary trial with different irrigation and organic manure additions, rice yields and other physiological features related to rice production, such as weight per thousand seeds, ear length and rice straw height, were affected to different extents (Table 7.2). The results indicated that the application of organic manure was important to maintain rice yield. Rice yields in the plots with

Table 7.2 Rice yields under different organic manure and irrigation practice

	With organic manure		Without organic manure	
	Non-flooded	Flooded	Non-flooded	Flooded
Yield (kg ha^{-1}, n=2)	8104.2	8114.6	6302.1	7197.9
Wt /1000 seeds (g, n=3)	27.5	27.5	28.6	29.0
Ear length (cm, n=10)	17.25	16.41	15.87	16.73
Straw height (cm, n=10)	95.28	97.41	82.25	86.51

organic manure were at least 10 % higher than that without organic manure. More importantly, if organic manure was applied before transplanting, rice yield would not be affected significantly by the difference in irrigation during the following rice growing season. Without organic manure application, rice yield was decreased by about 12 % in irrigation controlled plots than in the flooded plots.

Conclusions and Future Research Needed

Soil redox potential has an important impact on CH_4 and N_2O emission from rice fields. The trade-off relation of CH_4 and N_2O emissions with a change in soil redox potential has been demonstrated in the laboratory experiment of Chapter IV and field study of this Chapter. This inverse relation indicates the risk of stimulating N_2O production in attempting to reduce CH_4 production by increasing soil redox potential (e.g. when field is in drainage). Due to the same reason, it is difficult to control N_2O emission by keeping the soil reducing which is favorable for N_2 production during the denitrification process. Proper field management, such as control of irrigation and organic manure application should be developed that will minimize the emission of both of these greenhouse gases. Such a practice should keep the soil redox potential high enough to prevent CH_4 production, but not high enough to encourage N_2O production. Although the results in this study indicate that it is not practical to control the soil redox potential in an ideal range as in a laboratory study, proper management of irrigation and organic manure application can make a desirable change of soil redox conditions. The management option proposed from the results in this study is the controlled irrigation (non-flooded) with organic manure application. It provides a better chance to minimize

CH_4 and N_2O emissions from rice fields, and it does not have any adverse effect on rice growth and production.

Soil redox status is a good indicator of the soil environment regard to CH_4 and N_2O production and reduction. Therefore it would be very useful to determine soil redox potentials as part of different management practices. Further studies are needed to modify the management option proposed in this study to make it more effective and practical. A possible modification is to completely flood the fields immediately after inorganic fertilization, which may prevent short term vigorous N_2O production and emission. Effect of irrigation and organic manure application practice on rice plant aerenchyma formation and transport potential needs to be carefully evaluated, since transport through rice plant is the most important path of greenhouse gas emissions in rice fields. The season and amount of inorganic fertilizer applied need to be carefully determined, because nitrogen is likely becoming more moveable in non-flooded situation by conversion of ammonium to nitrate through nitrification activity. The long-term effects of such practices on soil properties and rice yield need to be investigated.

CHAPTER VIII IMBALANCE OF ATMOSPHERIC NITROUS OXIDE BUDGET AND FUTURE RESEARCH NEEDED

MASS IMBALANCE OF ATMOSPHERIC NITROUS OXIDE

Both estimated sources and sinks of N_2O are highly uncertain (see Appendix II). It is difficult in technique to quantitatively determine the biogenic fluxes of N_2O. This is caused by the extreme temporal and spatial variability of the processes of N_2O formation and exchange in all biogenic sources. The soil processes, nitrification and denitrification, are believed to account for up to 90 % of the atmospheric N_2O (Bouwman, 1990). Oxygen level in the soil has a fundamental effect on nitrification and denitrification activities and related N_2O release. The net production of N_2O by nitrifiers increases at low O_2 concentrations coupling with denitrification. Denitrification can be a net producer of N_2O under certain circumstances. The situation when O_2 concentration is slightly above zero, such as those found at the boundaries of suboxic zone, favors net production of N_2O under carbon-limiting conditions. In general, denitrifying bacteria can be net producers of N_2O in the early stages of denitrification before N_2O reductase has been synthesized in response to low O_2 condition. In early studies a few representative measurements were conducted and the results were used to extrapolate to the global N_2O flux. Recently more attention is paid to techniques of scaling. For example, terrestrial ecosystems should be stratified by delineation of functional types on the basis of soil, vegetation and terrain characteristics.

Denitrification consumes N_2O in suboxic zones and in anoxic environments where denitrification tends to complete with nitrogen gas as end product. As a matter of fact, this is the only known biological N_2O consumption mechanism. Soils may represent a

potential important sink of N_2O. There is still not enough data to evaluate the global consumption potential of atmospheric N_2O by soils. Current global estimates of emissions and atmospheric removal of N_2O do not account for other possible removal processes, such as uptake of N_2O by soils and the potential role of the ocean as a reservoir of N_2O. If other sinks of N_2O turn out to be important, the source estimates need to be revised as well to match the correct increase in N_2O concentration in the troposphere.

The global budget of N_2O shows a significant imbalance between the estimated rates of natural and anthropogenic production in soils and the ocean and the known rate of destruction in the stratosphere. An early study concluded that known and estimated inputs to the atmosphere are only about half of the flux required to balance the calculated destruction rate (Kim and Craig, 1993). Recent estimation still could not close the N_2O budget in the atmosphere (Appendix II). Despite the large uncertainties, there is a general agreement that the sources and sinks of the atmospheric N_2O can not be brought into agreement, and reasons for the imbalance are unknown. In view of these observations, more precise measurements need to be made on N_2O destruction in the stratosphere and that emitted from different terrestrial origins.

Isotopic Signature of Atmospheric Nitrous Oxide

Stable isotope data can provide both source-sink (tracer) and process (fractionation) information. Isotopic composition changes in a predictable way as an element cycles through the biosphere. These changes have been exploited by geochemists to understand the global elemental cycles. Isotopic fractionation in most biochemical reactions arises when similar molecules of slightly different mass react at

different rates. Stable isotopes are ideally suited to increase our understanding of element cycles in large ecosystems, such as N_2O study in a global perspective.

Multi-isotope characterization of N_2O emitted from various natural sources is a potentially powerful tool for providing the much-needed constraints. The use of stable isotopes as tracers in biogeochemical cycles is based on the presence of kinetic isotopic fractionation during biochemical processes. In these cases, molecules containing the heavier isotope react more slowly, the substrate becomes isotopically enriched, and the product is largely isotopically depleted so long as the substrate is not totally consumed (Note: when all substrate is converted to product in a complete reaction, there will be no opportunity for isotopic fractionation to occur). In soils and oceans, N_2O is formed as a by-product of nitrification and as an intermediary product of denitrification. In the latter process, N_2O can be further reduced to N_2. These processes, which operate on different source pools and have different magnitudes of isotopic fractionation, make separate contributions to the ^{15}N and ^{18}O isotopic composition of N_2O. In the case of nitrification in oxic waters, the isotopic composition of N_2O appears to depend mainly on the $^{15}N/^{14}N$ ratio of NH_4^+ and the $^{18}O/^{16}O$ ratio of O_2 and H_2O. In suboxic waters, denitrification causes progressive ^{15}N and ^{18}O enrichment of N_2O as a function of degree of depletion of nitrate and dissolved O_2. Nitrous oxide in soil and groundwater may be significantly depleted in ^{15}N and ^{18}O relative to tropospheric N_2O. Most of the studies in this area started from ocean. In surface ocean waters down to 600 m depth, N_2O is found depleted in both heavy isotopes, but at greater depth N_2O is enriched in ^{15}N and ^{18}O. It is generally believed that production of isotopically light N_2O (low $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios) occurs in the upper ocean through nitrification process, and that the flux of this light N_2O from

sea to air isotopically counters the flux of heavy N_2O from the stratosphere to the troposphere. However traditional paradigm for major sources of atmospheric N_2O cannot explain the isotopic composition of N_2O in the atmosphere, simply because the isotopic composition of N_2O from major terrestrial sources is significantly lighter than that of atmospheric N_2O, even after taking into account isotopic fractionation during destruction of N_2O in the stratosphere. More determinations of the isotopic ratios of N_2O in the atmosphere are apparently needed to identify and quantify N_2O sources.

Some research has suggested that the oceans could be an important source of isotopically enriched N_2O to the atmosphere (Kim and Craig, 1993). The most recent estimate of oceanic N_2O source (7-11 Tg N yr^{-1}) compares favorably with the biologically constrained figure (11 Tg N yr^{-1}). As these values approach the estimated stratospheric loss rate (12.3 Tg N yr^{-1}), they probably represent an upper limit (Dore et al., 1998). Soils are generally accepted to be the major source of atmospheric N_2O. It is likely that the role of the ocean in regulating the level and isotopic composition of atmospheric N_2O was over-estimated in their study. Even in their study case, air-sea exchange cannot, given the heavy isotopic signature of N_2O derived from the stratosphere, allow the tropospheric budget of N_2O to be closed without invoking hitherto-unknown N_2O sources and sinks. If the published data are representative, however, then there ought to exist some hitherto poorly known sources and/or sinks of N_2O that may be vital for tropospheric isotopic balance.

Possible Missing Sources and Sinks of Nitrous Oxide

The solubility of N_2O in water is relatively high (mole fraction solubility at 25 °C: N_2O 5.1×10^{-4}, CH_4 2.8×10^{-5}, CO_2 7.1×10^{-4}, Lide, 1991) which makes water flow a

possible important mechanism of N_2O transport and path of emission. Considerable N_2O fluxes may occur from surface water draining from fertilized agricultural fields (Dowdell et al., 1979) and even cleared forests (Bowden and Bormann, 1986), which account partially to the N_2O budget. A possible major missing source of N_2O may come from the emission through plant transpiration system. Most of the field measurements have been conducted using chamber technique, and the chamber size is commonly limited to cover the plant. Special case can be found in N_2O measurements from forest ecosystems where only floor soil can be covered by conventional chamber techniques. Transport of N_2O and CH_4 through rice plant has been studied and is considered as a major path of soil gases evolution to the atmosphere in rice field (Nouchi et al., 1990; Yu et al., 1997). Upland forests do not have such vascular transport system as in rice plants. However, soil gases can be released through plant transpiration system by dissolving in soil and plant fluids. Such potential is especially high for N_2O because it is such a soluble gas with the solubility in water close to that of CO_2. Thus, plants may potentially function as a conduit of N_2O from soil to the atmosphere through their transpiration system. If this hypothesis is valid, current estimation on N_2O source from forest ecosystem might be significantly underestimated, and N_2O emissions from other soil-plant system need to be re-evaluated. Such information will be profoundly important to correct the global N_2O budget.

It would be of great significance to conduct direct measurements of N_2O flux from plants and N_2O isotope composition. Information on isotopic ratios of N_2O will be helpful to close the atmospheric N_2O budget in isotope ratio. The $\delta^{15}N$ and $\delta^{18}O$ of

tropospheric N_2O is 7 ‰ and 20.7‰, respectively. The $^{18}O/^{16}O$ isotopic ratios of N_2O can be sensitively measured, and $\delta^{18}O$ values from N_2O derived from nitrification are lower than those for N_2O from denitrification. However, denitrification is the dominant mechanism for removal of fixed nitrogen from the biosphere. Recent studies on N_2O isotope analysis at different depth of ocean provide a useful insight (Naqvi et al., 1998). Denitrification produces substantial ^{15}N enrichment in subsurface nitrate, which is reflected in the isotopic composition of sinking particulate nitrogen. There is a general tendency for an inverse relationship between the $\delta^{15}N$ and $\delta^{18}O$ values of N_2O, and the O_2 concentration, indicating the important contribution of denitrification on N_2O production. Greater enrichment of ^{15}N in N_2O relative to nitrate appears to be characteristic of denitrification. The reduction process of N_2O to N_2 provides another opportunity to enrich ^{15}N of N_2O. Intense reducing conditions apparently cause the loss of N_2O to N_2, as manifested by the occurrence of very low N_2O concentrations, leaving the residual N_2O enriched with the heavier isotopes. In intensely reducing condition, the N_2O minimum observed coincided with an intense nitrite maximum and a pronounced peak of $\delta^{15}N$ of nitrate. Preferential loss of lighter N_2O to N_2 evidently led to enrichments of ^{15}N and ^{18}O in residual N_2O that are by far the greatest reported from any natural environment. The characteristics of N_2O concentration and corresponding isotope ratio in ocean could be as an analogue of that N_2O in vertical profile of soils. If same mechanisms of ^{15}N enrichments do occur in soils. Accumulation of heavier N_2O might exist in deeper layers of soil in prevalence of suboxic or anoxic environment. Nitrous oxide fluxes through plant transpiration process containing such ^{15}N enriched N_2O could be an important

source of tropospheric heavier N_2O. It will help to explain both the mass and isotope imbalance in tropospheric N_2O.

The question whether soil or ocean can function as a sink of N_2O remains unanswered. The reduction of N_2O to N_2 in denitrification process is a strictly anaerobic process. The microorganisms responsible for N_2O reduction are widely present in different ecosystems (Kromka et al., 1992; Okereke, 1993). The reducing environment can be found in flooded or deeper layers of soil. Nitrous oxide could also be consumed by aerobic soil, when an anaerobic environment is established in soil micro-zones (Ryden, 1981; Donoso et al., 1993). Thus, undisturbed soils might have a higher capacity of absorbing N_2O than disturbed soils. The capacity of soil to reduce N_2O greatly depends on soil nitrate content, because nitrate has been found to inhibit N_2O reduction activity in the mechanism of electron competition (Blackmer and Bremner, 1978; Letey et al., 1980; Terry and Tate III, 1980). Inhibition of the N_2O reduction to N_2 occurs at all NO_3 levels at low pH. At a higher soil pH the inhibition of N_2O reduction is temporary, although N_2O remains a significant product for a longer period at higher nitrate levels. Possibly a delay of N_2O reductase occurs until a threshold N_2O level is reached (Fillery, 1983). The fact that N_2O reduction enzyme can be induced in anaerobic environment suggests a higher potential of N_2O consumption by soils. Observations on consumption of atmospheric N_2O have been reported from field measurements of cultivated soils (Bremner et al., 1980; Yu et al., 1995; Mahmood et al., 1998), grasslands (Cicerone et al., 1978; Ryden, 1981; Donoso et al., 1993) and tropical soils (Keller et al., 1986; Matson and Vitousek, 1987). The N_2O reduction potential was shown to be higher in agricultural than in forest soil, and the capacity depended on several environmental parameters.

Favorable conditions for higher N_2O activity are anaerobic, pH near neutral and the nitrate content low. Wetlands are ideal sites to consume N_2O whenever nitrate content is at low level, because of their sustained anaerobic environment (Blicher and Hoffmann, 1999). Reduction of N_2O in soil is probably only a minor sink, but may still play an important role on a global scale. The elimination of N_2O in the stratosphere is so slow that even a small sink may contribute significantly to reducing the atmospheric residence time of N_2O.

FUTURE RESEARCH NEEDED

On spatial scales, knowledge of production location for both CH_4 and N_2O are needed, and they may not be the same part of the landscape for each gas (Cole et al., 1996). Because gas fluxes cannot be measured continuously in all locations within a region, some method of integration through time and space, modeling, is required. One approach is to examine the system processes and gas fluxes as a function of controlling factors through the use of simulation models to predict and extrapolate flux. These simulations can, ideally, reflect the interactions of the controlling variables on gas flux both spatially and temporally (Matson et al., 1989).

Methane has been comparatively well studied with most of the mechanisms of its production and emission widely documented. The broad picture of N_2O sources and sinks is less well documented and understood. This is partly due to the complex of the interactions of soils, oceanic subsurface and surface waters, stratospheric fluxes, and photolysis rates that determine the secular changes in the tropospheric concentration of N_2O. Some sources of N_2O emission have definitely been underestimated or undiscovered, because the current budget is far from balance. It is unknown if plant

transpiration is an actual N_2O transport mechanism, and if yes how much N_2O is emitted from this path. A new mechanism of N_2O production is a nitrification-denitrification coupling, where an intermediate or by-product of nitrification such as nitrite and NO may enter the denitrification sequence and become reduced to N_2O. The importance of denitrification also comes from its unique role in N_2O consumption potential. The increase of greenhouse gases' concentrations in the atmosphere is closely related to weakening of their sinks. The potential of soil and ocean as sink for atmospheric N_2O deserves attention in future attempts to estimate the atmospheric N_2O budget. A small increase in the sink strength will significantly contribute to reduce the residence time of N_2O in the atmosphere.

Isotopic analyses of gases collected under various sampling schemes should also help in assessing source intensities to various parts of a region. Stable isotope tracers are already present in natural systems, and their natural distribution reflects an integrated history of physical and metabolic processes within ecosystems. The major advantage of the stable isotope approach lies in field studies where measurements of existing isotopic distribution show how components of ecosystems are connected. The isotopic signature of N_2O should be a useful tool for studying the sources and sinks for N_2O in the biosphere and its impact on the atmosphere.

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APPENDIX I GLOBAL TROPOSPHERIC METHANE BUDGET

SOURCES	ESTIMATE	RANGE
	Tg y^{-1} (1 Tg = 10^{12} g)	
NATURAL		
Wetland	115	55-150
Termites	20	10-50
Ocean	15	5-50
Other	15	10-40
Subtotal	165	110-210
ANTHROPOGENIC		
Fossil fuel related	100	70-120
Animals (Enteric fermentation)	85	65-100
Rice paddies	60	20-100
Biomass burning	40	20-80
Landfill	40	20-70
Other	50	35-110
Subtotal	375	300-450
Total sources	540	410-660
SINKS		
Reaction with OH-radicals	500	400-600
Removal by soils	30	15-45
Total sinks	530	430-600
ATMOSPHERIC INCREASE	37	35-40

(Source: IPCC, 1995)

APPENDIX II GLOBAL TROPOSPHERIC NITROUS OXIDE BUDGET

SOURCES	ESTIMATE	RANGE
	Tg y^{-1} (1 Tg = 10^{12} g)	
NATURAL		
Ocean	3	1-5
Tropical soils		
wet forest	3	2.2-3.7
dry savannas	1	0.5-2.0
Temperate soils		
forests	1	0.1-2.0
grasslands	1	0.5-2.0
Subtotal	9	4.3-14.7
ANTHROPOGENIC		
Agricultural soils	3.5	1.8-5.3
Biomass burning	0.5	0.2-1.0
Industrial sources	1.3	0.7-1.8
Cattle and feedlots	0.4	0.2-0.5
Subtotal	5.7	3.7-7.7
Total sources	14.7	8-22.4
SINKS		
Stratospheric sink	12.3	9-16
Soils	?	?
Total sinks	12.3	9-16
ATMOSPHERIC INCREASE	3.9	3.1-4.7

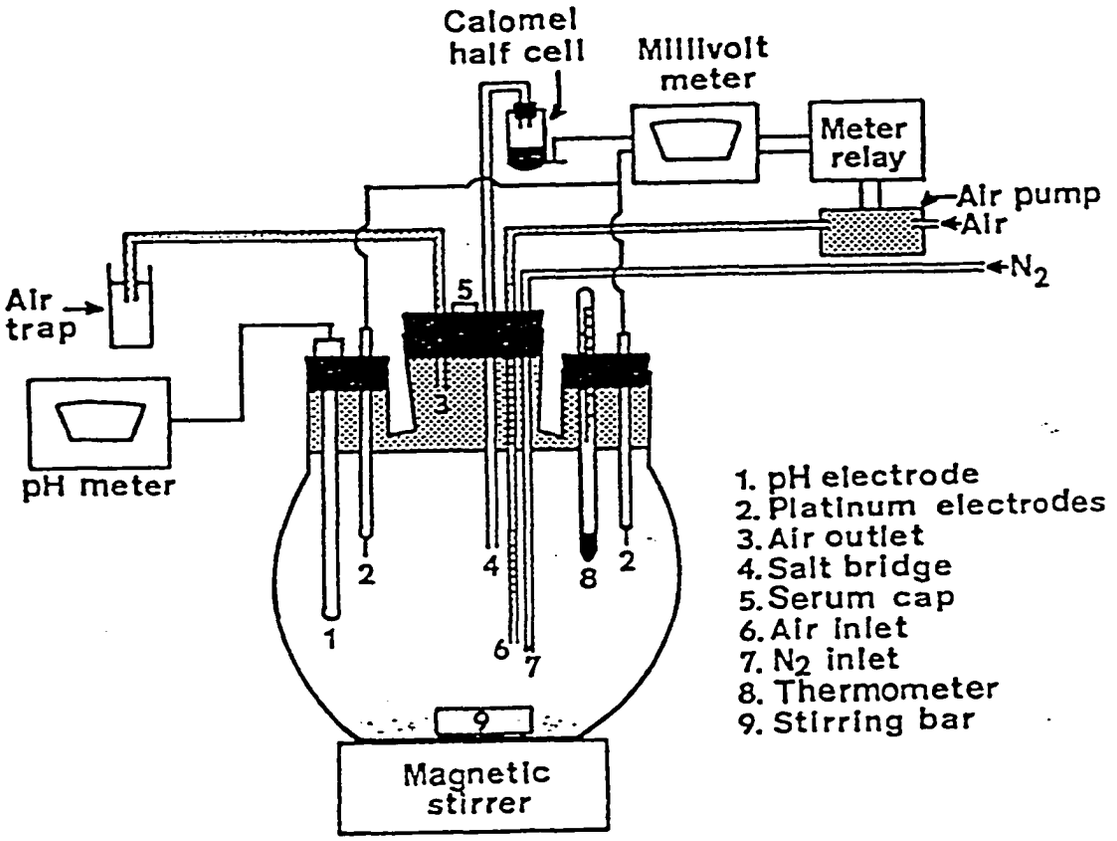
(Source: IPCC, 1995)

APPENDIX III MAIN CHARACTERISTICS OF THE SOILS USED IN THE STUDIES

Sample Soil	Texture	Organic Matter ($g\ kg^{-1}$)	Total Nitrogen ($g\ kg^{-1}$)	pH
US paddy soil	silt loam	15.7	0.80	5.7
Chinese paddy soil	clay loam	16.2	0.76	6.7
Belgian maize soil	loamy sand	35.3	1.60	6.0
Belgian wheat soil	silt loam	21.2	1.10	7.7

The US paddy soil was taken from the Rice Experiment Station, Crowley, Louisiana. The Chinese paddy soil was from the Shenyang Experimental Station of Ecology, Chinese Academy of Sciences. The maize and wheat soils were sampled from the Experimental Farm of the University of Ghent, Belgium. All four soils were air-dried, sieved (1 mm), and thoroughly mixed before use. We determined soil texture by the hydrometer method, soil organic matter by the dry combustion method (Nelson and Sommers, 1982), total Kjeldahl N by distillation (Bremner and Mulvaney, 1982), and pH by a pH electrode with soil:water ratio 1:2.

APPENDIX IV APPARATUS AND GENERAL PROCEDURE FOR SOIL SUSPENSION EXPERIMENT



The apparatus used for laboratory soil suspension study is called microcosm, a modification of the technique of Patrick et al. (1973). Four hundred grams of sample soil was weighed into a 2.3 l Erlenmeyer flask, to which 1.6 l of deionized water was added with remaining headspace volume of 0.55 l. The soil-water mixture was continuously stirred by a magnetic stirrer to make soil suspensions. Each flask was capped and sealed with a rubber stopper, in which a septum was installed for gas sampling. A gas inlet and outlet was installed in the stopper so that the accumulated gases in the headspace could be purged if needed. The redox potential of the soil suspensions was monitored by two Pt electrodes and a calomel reference electrode that were connected to a millivolt meter (Cole-Parmer, Illinois, USA). All the incubation experiments in this study were conducted at room temperature (25°C).

VITA

Kewei Yu was born on August 4, 1964, at Shenyang China. He entered Jilin University China in 1983, at the Department of Chemistry, and later the Department of Molecular Biology. He graduated in 1988 with bachelor of science degree in biochemistry. He continued his education at Graduate School and Institute of Applied Ecology, Chinese Academy of Sciences. He obtained a master of science degree in microbiology in 1991. He was employed as a research associate at the Institute of Applied Ecology, Chinese Academy of Sciences since his graduation, and was promoted to be a research assistant professor in 1994 and a research associate professor in 1998. He visited the University of Copenhagen for 3 month in 1994 to conduct an European Union project. He was granted a DANIDA fellowship from Danish Ministry of Foreign Affairs, so that he joined the research group in University of Copenhagen again in 1996.

He came to Louisiana State University to conduct a collaborative research project between Wetland Biogeochemistry Institute, LSU and Institute of Applied Ecology, Chinese Academy of Sciences. At the same time, he was enrolled in the doctoral study program at the Department of Oceanography and Coastal Sciences. He served as a research assistant of his major professor Dr. William H. Patrick for 3 years. He involved in three research projects during this period of time, funded by Louisiana Board of Regent, by NATO in cooperation with Belgium, and by USDA in cooperation with China, respectively. He has more than 30 publications in refereed journal articles, book chapters, and presentations at scientific meetings. He will be awarded the degree of Doctor of Philosophy at the December 2000 Commencement.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

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Major Field: Oceanography and Coastal Sciences

Title of Dissertation: Oxidation-Reduction Chemistry of Methane and Nitrous Oxide in Soils and an Approach to Minimize Their Emissions from Irrigated Rice Fields

Approved:

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Date of Examination:

October 30, 2000